International Journal of Reproduction, Contraception, Obstetrics and Gynecology
Nayak NR et al. Int J Reprod Contracept Obstet Gynecol. 2020 May;9(5):1794-1799
www.ijrcog.org

pISSN 2320-1770 | eISSN 2320-1789

DOI: http://dx.doi.org/10.18203/2320-1770.ijrcog20201527

Original Research Article

Retrospective analysis of intrauterine granulocyte colony-stimulating factor in controlled ovarian stimulation with intrauterine insemination cycle

Navinchandra R. Nayak*, Madhumeetha T., Mangala Gowri Hegde, Aparna C. Aravind, Ritu Sharma A., Shwetha Ballal K.

Department of Obstetrics and Gynecology, AJ Institute of Medical Sciences and Research Centre, Mangalore, Karnataka, India

Received: 19 March 2020
Accepted: 30 March 2020

***Correspondence:**
Dr. Navinchandra R. Nayak,
E-mail: nayak66@yahoo.co.in

ABSTRACT

Background: Persistent thin endometrium affects <1% of patients. Various treatments have been proposed with no satisfactory results. GCSF is one such treatment modality which improves endometrial thickness and implantation. Aim of this study was to analyse the effects of dose and the site of instilling intrauterine G-CSF in COS IUI cycles in patients with unexplained infertility and to note the pregnancy rates among them.
Methods: It is a 3-year retrospective study done in obstetrics and gynecology department of AJ Institute of Medical Sciences and Research Centre, that included all unexplained infertility cycles with controlled ovulation stimulation-IUI protocols where for a thin endometrium GCSF was used. The method of ovarian stimulation, the drug and dose used, the trigger for ovulation and the ovarian and endometrial response was noted. The day of the intrauterine GCSF and the dose and the site of instillation was noted. The endometrial response to GCSF the outcome for pregnancy was noted. All the data was analyzed statistically.
Results: Significant endometrial response was seen with a dose of 100 mg,150 mg and 300 mg. Pregnancy outcome was better when GCSF was instilled just above the level of the os. GCSF instilled at the level of the fundus increases the possibility of ectopic pregnancy.
Conclusions: Instillation of GCSF of 100 mg dosage just above the os; is a safe and effective method for improving the endometrial thickness and increasing pregnancy rate.

Keywords: Endometrial thickness, Granulocyte colony stimulating factor, Intra uterine insemination, Pregnancy outcome, Unexplained infertility

INTRODUCTION

Embryo implantation largely depends on the quality of the embryo and the endometrial receptivity. It is estimated that implantation failure is responsible for approximately 50% to 75% of lost pregnancies.[1] Despite major advancements in assisted reproductive techniques, the implantation rates remain relatively low. "Successful implantation requires good quality of embryos, receptive endometrium, and proper embryo transfer technique".[2]

The receptive endometrium is defined as a healthy uterine milieu which support the transformation of endometrial cells into decidual cells, invasion of blastocysts, and growth of placenta.[2] This mechanism is helped by immune cells, growth factors, cytokines, and hormonal changes. Immunological mechanisms in the endometrium are very crucial and important in implantation.[1]

The normal thickness of endometrium is 7 to 14 mm in the secretory phase and it is a prominent factor for

successful pregnancy. Studies show that pregnancy doesn't occur if endometrium thickness is less than 6 mm.[2,3] Persistent thin endometrium resistant to standard treatments affects <1% of patients and is really a frustrating problem. Thin endometrium remains a challenge in gynecology and reproductive science with only slight enhancements attained with the currently available treatment. The probable causes for thin endometrium are endometrial resistance to estrogen, impaired sub-endometrial blood flow, damage to basal endometrium following vigorous curettage and Asherman syndrome. Thin endometrium also carries a high risk for miscarriage.[4]

Various treatments have been proposed, including extended estrogen administration, low-dose aspirin, pentoxifylline, tocopherol, and vaginal sildenafil citrate, but have found to be ineffective.[5] Alternative treatments like tocopherol and pentoxifylline increase endometrial thickness and pregnancy outcome but length of treatment period ought to be 6-9 months.[3] Other modes of treatment like HCG, endometrial progenitor cells, platelet rich plasma, bone marrow stem cells and mesenchymal progenitor cells are in evaluation for improvement of endometrial thickness.[6]

Granulocyte colony-stimulating factor (G-CSF) is a hematopoietic cytokine that appears in the materno-fetal interface during embryo implantation and early pregnancy suggesting it may have a role in decidua and placental development.[6] It enhances granulocyte proliferation and differentiation. Hence it improves implantation. It also affects human decidual macrophages, ovulation, granulosa cell function and improves ovarian stimulation in poor responders.[5,7]

G-CSF is predictive of IVF outcome, it is a biomarker for oocyte/embryos with implantation potential, reduces unexplained repeated pregnancy loss and plays a role in the genesis of early endometriotic lesions, and suppresses autoimmunity.[8-10] Administration of G-CSF does not seem to affect embryonic chromosomal constitution and therefore seems safe. Some studies have shown that systemic administration of G-CSF in patients with recurrent spontaneous pregnancy losses and repetitive implantation failures improves pregnancy outcomes.[6-8] Also, studies show that transvaginal infusion of GCSF successfully were utilized in women with low endometrial thickness and recurrent implantation failures.[5,9,11] So, it is proven that G-CSF's inflammatory and immunological effects improves the implantation rate and endometrial receptivity in infertile women.[12]

GCSF also has adverse effects such as skin rash, injection site rash, bone pain and myalgia but this is seen with the use of GCSF for haematological purposes. In intrauterine administration not much effects have been reported. GCSF also does not show any improvement in older age group women.[5,6]

This study was conducted with an objective to analyse the effects of dose and the site of instilling intrauterine GCSF in COS IUI cycles in those for unexplained infertility and to note the pregnancy rates among them.

METHODS

It is a 3-year retrospective study done in OBG department of AJ Institute of Medical Sciences and Research Centre from January 2017 to January 2020, that included all unexplained infertility cycles with controlled ovulation stimulation-IUI protocols where for a thin endometrium GCSF was used.

Data from the records; regarding their history including age, BMI, duration of infertility, basic infertility evaluation results, report of last ultrasound, obstetric history, marital status, previous abdominal surgery, history of disease, and pharmacological treatments of all women with the inclusive criteria who had consented for GCSF administered IUI was collected.

The method of ovarian stimulation, the drug and dose used, the trigger for ovulation and the ovarian and endometrial response was noted. The day of the intrauterine GCSF and the dose and the site of instillation was noted. The endometrial response to GCSF, the outcome for pregnancy was noted. All the data was analysed statistically.

Inclusion criteria

- Women with unexplained infertility. In this study defined unexplained infertility as a couple who have had regular unprotected intercourse for 1 year (or 6 months when the female partner is more than 35 years) and all tests of basic infertility including semen analysis within normal limits, with a documentation of regular ovulation and documentation of normal uterus with patent tubes[2]
- Women with thin endometrium. Thin endometrium was defined as endometrial thickness less than 7mm on transvaginal ultrasound[2]
- IUI cycles.

Exclusion criteria

- Any medical or surgical illness
- Known causes of infertility
- Abnormal semen parameters
- Cycles with gonadotrophins are not taken into account.

Statistical analysis

Data was entered into Microsoft excel data sheet and was analyzed using SPSS 22 version software. Categorical data was represented in the form of Frequencies and proportions. Chi-square test was used as test of significance for qualitative data. Continuous data was

represented as mean and standard deviation. Paired t test is the test of significance for paired data such as before and after surgery for quantitative data. p value (probability that the result is true) of <0.05 was considered as statistically significant after assuming all the rules of statistical tests.

RESULTS

In the study majority of women were in the age group of 26 to 30 years and majority of husbands were in the age group 30 to 35 years. It was observed that all the patients had undergone a baseline transvaginal ultrasound to determine their antral follicle count on day 2 of their cycle and based on the antral follicle count, Clomiphene citrate in strengths of 100 mg or 50 mg or Letrozole in the strength of 2.5 mg or 5 mg from day 2 to 5 was used for ovulation stimulation. No additional gonadotropins were used in the rest of the course of treatment.

It was also observed that arginine sachets was given on 6th hourly basis for a period of 3-5 days prior to ovulation trigger in 23 patients. Ovulation trigger was done using injection HCG and inj. leuprolide. On the day of the trigger, it was observed that G-CSF injection at doses of 300 mg/ 150 mg/ 100 mg had been instilled into the uterine cavity at the level of the fundus or the mid cavity or just above the internal os using an intrauterine insemination catheter. Post 34-36 hours; IUI was done under aseptic precautions. The patients were reviewed after 16 days for serum β-hCG levels.

A total of 59 subjects were included of which 45 patients received injection GCSF 100 mg. Among them GCSF was instilled just above the level of the os in 40 (88%) patients and at the level of fundus in 5 (11%) patients. In 3 of the patient's injection GCSF of 150 mg was instilled in the mid cavity. In 11 patients; injection GCSF of 300 mg was instilled just above the level of the os in 3 (27%) and at the fundus in 8 (72%) patients (Table 1).

Table 1: General profile of subjects in the study.

		Site of injection					
		Above internal os		At mid cavity		At fundus	
		Count	%	Count	%	Count	%
Inj. GCSF dose	100 mg	40	88.0%	0	0.0%	5	11.0%
	150 mg	0	0.0%	3	100.0%	0	0.0%
	300 mg	3	27.0%	0	0.0%	8	72.0%

Table 2: ET comparison before and after GCSF with respect to dose and at different sites.

	Inj GCSF 100 mg			Inj GCSF 150 mg			Inj GCSF 300 mg		
	ET before GCSF	ET after GCSF	p value	ET before GCSF	ET after GCSF	p value	ET before GCSF	ET after GCSF	p value
Above internal os	5.75±0.27	7.87±0.64	<0.001*				5.77±0.35	8.93±1.01	0.014*
At mid cavity				5.97±0.15	7.67±0.50	0.046*			
At fundus	5.47±0.31	8.03±0.78	<0.001*				5.86±0.59	7.85±0.59	<0.001*

*ET- Endometrial thickness.

Table 3: ET comparison before and after GCSF with respect to site of Inj. GCSF.

		ET before GCSF		ET after GCSF		p value
		Mean	SD	Mean	SD	
Site of inj. GCF	Above internal os	5.75	0.27	7.95	0.71	<0.001*
	At mid cavity	5.97	0.15	7.67	0.50	0.046*
	At fundus	5.69	0.52	7.93	0.65	<0.001*

*ET- Endometrial thickness.

It was observed that in the study, there was significant increase in ET after instilling injection GCSF compared to before the injection in all the groups (Table 2). The mean increase in ET for 100 mg of GCSF above the level of OS was 7.87 (p value <0.001) and at the level of fundus was 8.93 (p value <0.001). The mean increase in ET for 150 mg of GCSF was 7.67 (p value-0.046) at mid cavity. Similarly, the mean increase in ET for 300 mg GCSF at the level of os was 8.03 (p value <0.001) and at the level of fundus was 7.85 (p value < 0.001). It was

observed that there was significant increase in ET after GCSF in all the groups i.e. at the level above the internal os, at mid cavity, and at fundus irrespective of the dosage. And comparatively the mean increase in ET was more in the above the internal os group 7.95 (p value <0.001) when compared with other two groups (Table 3).

Table 4: ET Comparison before and after GCSF at different doses.

		ET before GCSF		ET after GCSF		p value
		Mean	SD	Mean	SD	
Dose	100 mg	5.71	0.29	7.90	0.66	<0.001*
	150 mg	5.97	0.15	7.67	0.50	0.046*
	300 mg	5.84	0.52	8.15	0.84	<0.001*

*ET- Endometrial thickness.

Table 5: Comparison of site of injection with outcome of IUI with respect to dose of injection GCSF.

Site of inj. GCF	Dose											
	100 mg				150 mg				300 mg			
	Outcome of IUI				Outcome of IUI				Outcome of IUI			
	Failed iui	Pregnancy	Missed abortion	Ruptured ectopic	Failed IUI	Pregnancy	Missed abortion	Ruptured ectopic	Failed IUI	Pregnancy	Missed abortion	Ruptured ectopic
Above internal os	31	9	0	0	0	0	0	0	2	0	1	0
At mid cavity	0	0	0	0	2	1	0	0	0	0	0	0
At fundus	3	2	1	0	0	0	0	0	6	0	0	2
p value	0.024*				-				0.179			

In the study there was a significant increase in mean ET at all the doses irrespective of the site of the injection. It was noted that the mean increase in ET was high in subjects who received 300 mg of Inj. GCSF (Table 4).

On comparison of the outcome (Table 5) it was observed that among those who received 100 mg of Inj GCSF above the internal os; 9 patients became pregnant and the remaining 31 had a failed IUI. And with 100 mg at the level of the fundus; 2 patients had a failed IUI and 1 had a missed abortion. Among those who received 150 mg of inj GCSF at the level of the mid cavity; 1 patient had become pregnant and 2 had a failed IUI. Similarly, among the patients who received 300 mg of Inj. GCSF above the internal os; 2 became pregnant and 3 had a failed IUI and 1 patient had a missed abortion and with 300 mg of inj. GCSF at the fundus; 2 patients had ruptured ectopic gestation and 6 patients had a failed IUI.

DISCUSSION

GCSF demonstrates divergent roles in reproduction, having distinct effects on endometrium and implantation. A potentially growth expanding effect on endometrium may be suspected from its role in establishing early endometriotic lesions. Thus, in women presenting with unexplained infertility with a thin endometrium, granulocyte colony stimulating factor (GCSF) can be used to acquire a desirable reproductive outcome. It is also proven through several studies that GCSF when used for recurrent spontaneous pregnancy losses and repetitive implantation failures improves pregnancy outcomes.

In animal models GCSF of >130 pg/ml was associated with successful implantation rates. Various doses have been studied for instillation of GCSF, studies like Gleicher et al, Kuniki et al have demonstrated improvement in endometrial thickness with a dose of 300 mcg given on 14th day (day of HCG administration), while another study Li et el did not show any improvement in thickness with 100 mcg of GCSF.[5,13,14] In this study it was observed that a significant increase in endometrial thickness was noted in all 3 dosage groups irrespective of the level of instillation of GCSF (Table 4). It was noted that the mean increase in ET was high in subjects who received 300 mg above the internal os. In the study by Gleicher et al, the site of instillation was studied which suggested that higher instillation (near the fundus of uterine cavity) and larger volume could cause leakage into the tubes and possible ectopic pregnancy and hence instillation of approximately 1 ml into the mid uterine cavity just above the internal os was suggested. In this study there was significant increase in ET after GCSF in all the sites i.e. above the internal os, at midcavity, and at the fundus irrespective of the dosage.[5] (Table 3) and comparatively the mean increase in ET was

more in the above the internal os group when compared with other two groups.

In this study there was significant association noted between outcome of IUI and site of inj. GCSF. Among those who received 100 mg of GCSF above the internal os; 9 patients who underwent IUI became pregnant. And at the level of the mid cavity with 150 mg of GCSF 1 patient had become pregnant and 2 had a failed IUI. Similarly, among the patients who received 300 mg of inj. GCSF above the internal os; 2 became pregnant and 3 had a failed IUI and 1 patient had a missed abortion and with 300 mg at the fundus; 2 patients had a ruptured ectopic gestation and 6 patients had a failed IUI. These findings are similar to other studies.[5,9] Hence from this study it is observed that injection GCSF when given at the level of fundus caused an increase in the incidence of ectopic gestation probably because of the spillage into the tubes from fundus. Only 1 patient had missed abortion when GCSF was instilled above the level of the internal os. Thus, we could state that injection GCSF can be safely administered above the level of the os for improvement of endometrial thickness.

On comparing the dose of Inj GCSF with site of instillation; (Table 2) the mean increase in ET for 100 mg of GSF above the level of OS was 7.87 (p value <0.001) and at the level of fundus was 8.93 (p value <0.001). The mean increase in ET for 150 mg of GCSF was 7.67 (p value-0.046) at mid cavity. The mean increase in ET for 300mg GCSF at the level of os was 8.03 (p value <0.001) and at the level of fundus was 7.85 (p value < 0.001). Hence it was noted that significant increase in endometrial thickness among the 3 dosage groups was in the 300 mg of GCSF group above the level of internal os. Comparison of the dosages and the outcomes with various dosages was not done in other studies making this study a first to compare the various dosages of GCSF with the various sites and assessing their outcomes.

Although the mean increase in ET was more with the 300 mg group (mean increase above the level of internal os-8.73) when compared with the other two dosage groups. With 300 mg dosage the complications like ruptured ectopic and missed abortion was also more among the 300 mg group patients. Even at the level above the internal os in the 300 mg group 1 out of the 3 patients had a missed abortion. Among the 100 mg group of patients only 1 patient in whom GCSF was instilled at the fundus had a missed abortion. Therefore, study could state that from this study 100 mg of injection GCSF can be safely administered at a level just above the internal os for improving the endometrial thickness.

CONCLUSION

Infusion of G-CSF in endometrial cavity is a safe and probably effective method to increasing endometrial thickness for patients with thin and unresponsive endometrium.

Hence from this study we conclude that injection GCSF for improvement of endometrial thickness and pregnancy rate; can be safely administered at a dose of 100 mg at a level just above the internal os.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. Sarvi F, Arabahmadi M, Alleyassin A, Aghahosseini M, Ghasemi M. 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. Obstet Gynecol Int. 2017;3596079.
2. Speroff L, Fritz MA, editors. Clinical gynecologic endocrinology and infertility. lippincott Williams & wilkins; section 4; Speroff. 8th edition; 2005:185:1167.
3. Samara N, Bentov Y. Current Strategies to Manage a Thin Endometrium. Womens Health Gynecol. 2016;2(4):003e.
4. Eftekhar M, Hosseinisadat R, Baradaran R, Naghshineh E. Effect of granulocyte colony stimulating factor (G-CSF) on IVF outcomes in infertile women: An RCT. Inter J Reprod BioMed. 2016;14(5):341.
5. Gleicher N, Vidali A, Barad DH. Successful treatment of unresponsive thin endometrium. Fertil Steril. 2011;95(6):2123-e13.
6. Kamath MS, Lele P. Granulocyte colony stimulating factor for treatment of thin endometrium in assisted reproduction technology cycles. Int J Infertil Fetal Med. 2015;6(3):97-102.
7. Barad DH, Yu Y, Kushnir VA, Shohat-Tal A, Lazzaroni E, Lee HJ, ET AL. 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.
8. Tehraninejad E, Tanha FD, Asadi E, Kamali K, Aziminikoo E, Rezayof E. G-CSF intrauterine for thin endometrium, and pregnancy outcome. J Family Reprod Health. 2015;9(3):107.
9. Mishra VV, 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. The J Obstet Gynecol India. 2016;66(1):407-11.
10. Eftekhar M, Tabibnejad N, Tabatabaie AA. The thin endometrium in assisted reproductive technology: an ongoing challenge. Middle East Fertil Society J. 2018;23(1):1-7.
11. Eftekhar M, Sayadi M, Arabjahvani F. Transvaginal perfusion of G-CSF for infertile women with thin

endometrium in frozen ET program: A non-randomized clinical trial. Iranian J Reprod Med. 2014;12(10):661.
12. Aleyasin A, Abediasl Z, Nazari A, Sheikh M. Granulocyte colony-stimulating factor in repeated IVF failure, a randomized trial. Reprod. 2016;151(6):637-42.
13. 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 Inter. 2014;2014.
14. Zhang L, Xu WH, Fu XH, Huang QX, Guo XY, Zhang L, et al. Therapeutic role of granulocyte colony-stimulating factor (G-CSF) for infertile women under in vitro fertilization and embryo transfer (IVF-ET) treatment: a meta-analysis. Arch Gynecol Obstet. 2018;298(5):861-71.

Cite this article as: Nayak NR, Madhumeetha T, Hegde MG, Aravind AC, Sharma RA, Ballal SK. Retrospective analysis of intrauterine granulocyte colony-stimulating factor in controlled ovarian stimulation with intrauterine insemination cycle. Int J Reprod Contracept Obstet Gynecol 2020;9:1794-9.