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Can Granulocyte Colony Stimulating Factor Infusion Further Improve the Pregnancy Rate Even with Good Endometrial Thickness in a Frozen Embryo Transfer Cycle?

Nikita N[1]*, Rajesh S[1] and Amit A[2]

1 Assisted Reproductive Technology Centre, Command Hospital, Pune, India
2 Urologist Command Hospital, Pune, India

***Corresponding author:** Nikita Naredi

Art Specialist, Assisted Reproductive Technology Centre, Command Hospital, Pune 410040, India.

nikitanaredi@gmail.com

Tel: 08800504222

Abstract

Background: Use of Granulocyte Colony Stimulating factor (G-CSF) has been in vogue for almost half a decade in Assisted Reproductive Technology for its role in improving unresponsive endometrium. In addition various studies postulates its additional role in improving implantation in infertile women as GCSF receptors have been found to play an important role in female reproduction.

Objective: To assess if intrauterine G-CSF infusion improves the pregnancy rates in women with normal endometrial thickness undergoing frozen embryo transfer cycle (FET).

Keywords: G-CSF; Frozen embryo transfer; Pregnancy rate

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Introduction

Endometrium which was a neglected entity till a decade back in assisted reproduction has drawn a great focus in recent years with emphasis on factors like endometrial morphology, receptivity and window of implantation to enhance the success of an in vitro fertilisation (IVF) cycle, be it after a fresh transfer or a frozen embryo transfer (FET). However to achieve an implantation and in turn an enhanced clinical pregnancy rate a synergy is required between a top quality embryo and a receptive endometrium. It has been postulated by various studies that 2/3 implantation failure is due to poor endometrial receptivity [1,2]. Many workers have thus proven that endometrial thickness is an important index which assesses the endometrial receptivity and that endometrial thickness (ET) below a cut-off leads to implantation failure and in turn a reduced pregnancy rate [3-6]. In pursuance various drugs have been used for enhancement of endometrial thickness like extended estrogen administration, low-dose aspirin, combination of pentoxifylline and tocopherol, vaginal sildenafil citrate, and the latest being Granulocyte Colony stimulating factor infusion (G-CSF) [7-14]. These treatment modalities have had mixed results with some showing improved endometrial receptivity and increased implantation and pregnancy rate in ART cycles while few documenting no response.

Granulocyte colony stimulating factor, a glycoprotein of the growth factor family which was first successfully administered by Gleicher and his co-workers in 2012 for treating an unresponsive endometrium has come in a big way in the armamentarium of the reproductive specialists to improve the ET and possibly improve the implantation rates [13,14]. In spite of the initial success with G-CSF various studies have given conflicting results on the outcome of assisted reproductive technology (ART) cycles with thin endometrium or recurrent implantation failure (RIF) [15,16]. Nevertheless G-CSF has been elucidated to be present in the female reproductive system and play a putative role in human reproduction with impact on follicular development, ovulation, ovarian response to stimulation, and establishment and maintenance of pregnancy [17,18]. It has also been observed that G-CSF increases the cAMP-mediated decidualization of human endometrial stromal cells in an autocrine or paracrine fashion [17,18].

With the background knowledge that G-CSF and G-CSF receptors are present in the endometrium, decidua and placenta, we aimed to carry out a study to investigate if intrauterine infusion of G-CSF further improved the pregnancy rate in a frozen embryo transfer cycle. The novelty of our study is that we carried out

G-CSF administration in women with an adequate endometrial thickness i.e., >7 mm.

Material and Methods

Study design and subject selection

This was a prospective non randomized controlled trial, involving patients undergoing frozen embryo transfer after IVF treatment at the Assisted reproductive technology centre of a tertiary care hospital. The subjects were recruited from the period between January 2015 and August 2016. Approval for the study was obtained from the Institutional Review Board.

Study participants

The patients were enrolled for the study only if their age was less than or equal to 38 years and excluded if they had any of the factors having a detrimental impact on implantation like: presence of intramural fibroid distorting the endometrial cavity/submucous myoma/Asherman's syndrome, presence of sonographically detected hydrosalpinx, history of any uterine surgery in the past: metroplasty, septoplasty etc. They were also not a part of the study if the endometrial thickness was less than 7 mm, had a history of recurrent implantation failure (failure to conceive following two embryo transfer cycles) or with a known congenital uterine anomaly or use of any other drugs other than estrogen for endometrial preparation.

The reasons for carrying frozen embryo transfer in the present study was: failed fresh IVF cycle, at risk for ovarian hyperstimulation syndrome for which a "freeze all" strategy was used, presence of endometrial polyps detected during stimulation cycle which was subsequently removed, poor endometrial thickness during the IVF cycle, increased progesterone levels >1.0 ng/mL on the day of ovulation trigger and few others like patients preference, poor medical condition of the patient during fresh transfer, inability to negotiate the cervical canal.

We chose a retrospective cohort from the data registry of our IVF centre as the control group. This Group II comprised of the first 75 patients who underwent frozen embryo transfer without G-CSF administration at our centre during the study period before G-CSF was introduced as part of the treatment protocol, and met the same inclusion and exclusion criteria. Women who were administered G-CSF belonged to Group I.

A total of 75 women met the inclusion and exclusion criteria who were administered G-CSF during their frozen embryo transfer cycle. Thus to make the study comparable data of 75 women were taken from the retrospective cohort of frozen cycles who also met the inclusion and exclusion criteria.

Endometrial preparation

All women (both control and study group) received Estradiol Valerate tablet (2 mg, Progynova; Bayer Zydus) 6 mg daily from the second day of the menstrual cycle after doing a baseline day 2 transvaginal ultrasound (TVS). Ultrasonography was done from the 12th-13th day of cycle. Endometrial thickness was measured at its thickest part in the longitudinal axis of the uterus. When the endometrial thickness was more than 7 mm, the same sonologist measured it repeatedly for 2 times to confirm the endometrial thickness, and the average value of the 2 different measurements was recorded.

G CSF Infusion and progesterone support

The day the endometrial thickness was more than 7 mm the G-CSF group underwent intrauterine infusion of 300 µg (300 mcg/1 mL) of G-CSF. Before infusion, the content of the ampoule was aspirated into a 1 mL insulin syringe, the intra uterine insemination (IUI) cannula was introduced into the endometrial cavity as performed during an intrauterine insemination and the content of the syringe was slowly injected into the cavity. Once the content was completely injected, a small amount of air was aspirated after disconnecting the syringe and then reconnected to the catheter and the air bubble was injected, to pass the remnant amount of G-CSG left in the (IUI) catheter into the endometrial cavity.

The progesterone support was as per the institutional protocol for both the groups: injection micronized progesterone 100 mg intramuscularly along with tablet dydrogeterone 10 mg twice a day (Duphaston; Solvay). Estradiol Valerate and progesterone support was continued even after embryo transfer till BHCG report was available and further thereafter if the report was positive.

Embryo transfer

A repeat endometrial measurement was made on the day of embryo transfer for Group I women. Endometrial thickness was always measured at the thickest point. After warming the embryos as per the protocol, two grade I embryos at 8 cell stage were transferred under transabdominal ultrasound guidance on day 3.

Pregnancy outcome

Pregnancy outcome was assessed based on positive serum βhCG assay (chemical pregnancy), 14 days after embryo transfer. Values greater 50 miu/ml was taken as positive βhCG and observation of a gestational sac on transvaginal ultrasound examination as clinical pregnancy, 17 days after embryo transfer. The ongoing pregnancy rate was defined as the presence of foetal cardiac activity by ultrasonography after 12 weeks of pregnancy.

Statistical analysis

Data were analyzed using Statistical Package for the Social sciences 20.0 (SPSS, SPSS Inc, Chicago, Illinois). Continuous data were presented as mean ± SD and assessed by independent Student's T-test. Qualitative data were compared by Fisher exact test. $P<0.05$ was considered significant.

Results

A total of 150 patients were studied of which 75 belonged to group I i.e., the G-CSF treated group and 75 were in the control group i.e., who were not exhibited G-CSF.

The baseline and hormonal profile of the patients have been presented in **Tables 1 and 2**. Both the study group women were comparable in terms of age, body mass index (BMI) attempt at FET cycle, duration of infertility and the cause of sub fertility. Their hormonal profile was also similar in terms of serum follicle stimulating hormone (FSH), luteinising hormone (LH) and antimullerian hormone (AMH) values.

The reason for carrying out frozen embryo transfer in the two study groups were also comparable with maximum cycles being done due to a failed fresh IVF-ET cycle **(Table 3).**

The mean endometrial thickness in both the groups was same and it was also observed that there was no increase in the thickness (8.1 vs. 8.18) of the endometrium in women who received G-CSF.

The pregnancy rate of the G-CSF group was 52% (39/75) in comparison to the pregnancy rate of 45.33% (34/75) in the control group. Although group I had more βhCG positives as compared to group II it was not statistically significant **(Table 4)**.

Table 1 Base line and hormonal profile of the study groups.

	GCSF treated N=75	Non GCSF Group N=75	P value
Average age (in years)	28.9 ±2.88	28±2.74	0.59
Average attempt	1.65± 0.60	1.57±0.59	0.20
Average BMI kg/m2	22.9 ±2.62	22.8±2.39	037
Average FSH (IU/L)	5.27±3.75	5±3.76	0.38
Average LH (IU/L)	6.19 ±2.43	5.9±2.29	0.28
Average AMH	2.87	2.6	0.19
Endometrial thickness(mm)	8.18	8.1	0.5

Table 2 Cause of infertility in the two groups.

Cause of Infertility (%)	Group I (with GCSF)	Group II (Non GCSF)	P value
Male	21	23.6	0.349
Tubal	31.50	28.9	0.363
Ovarian	19.70	18.4	0.418
Uterine	19.70	6.5	0.346
Unexplained	7.80	22.3	0.378

Table 3 Reasons for carrying out frozen embryo transfer cycle.

Reason for FET	Group I (with GCSF)	Group II (Non GCSF)	*P value*
Poor ET	16.60	22.3	*0.5*
Failed previous ET	30.70	31.5	*0.431*
OHSS risk	15.30	15.7	*0.414*
Endometrial Polyps in fresh cycle	11.50	11.8	*0.316*
Increased Progesterone pre hCG trigger	9.00	9.2	*0.299*
Others	17.90	11.8	*0.789*

Table 4 Change in endometrial thickness.

	Pre GCSF	Post GCSF	P value
Average ET	8.10	8.18	0.257

Discussion

Chronically thin endometrium resistant to standard treatment which was posing a challenge to the IVF specialists worldwide seemed to have been overcome by Gleicher et al in 2011 [14]. When they published the successful story of granulocyte colony-stimulating factor (G-CSF) in improving the endometrial thickness of four women who failed to show any response to any of the available modalities to improve the ET during their IVF cycles. The saga of G-CSF since then has been surrounded by controversies whether it is about its efficacy in improving endometrial thickness, the route of administration, improvement in implantation and pregnancy rates or its overall role in human reproduction.

Despite the controversies on its efficiency and efficacy, this glycoprotein has been postulated to have growth factor and cytokine functions which help in the proliferation and differentiation of normal human endometrial cells [17]. Various researchers have also elucidated that G-CSF is an important component in female reproductive system playing roles at all levels be it follicular development, ovulation, ovarian response to stimulation, or establishment and maintenance of pregnancy [19-21]. This was further brought out very recently in the systematic review and meta analysis by Zhao et al in which they concluded that the pregnancy rate with G-CSF administration was significantly higher compared with cases without G-CSF administration although there was no increased embryo implantation rate in G-CSF administration cycles [22]. In our study too, the pregnancy rate of the G-CSF group was 52% (39/75) in comparison to the pregnancy rate of 45% (34/75) in the control group with group I having more βhCG positives as compared to group II but it was not statistically significant. However in our study the route of administration was intrauterine as opposed to studies in which G-CSF administrated subcutaneously resulted in significantly higher pregnancy rates [22].

Eftekhar et al in their observation on IVF outcome after G-CSF infusion did not find any improvement in pregnancy outcomes in terms of chemical, clinical and ongoing pregnancy rate, implantation rate, and miscarriage rate [23]. Their study similar to the present work assessed the pregnancy outcome after removing the rate limiting factor of endometrial thickness with the only difference being that Eftekhar et al studied a fresh IVF cycle in contrast to ours which was on a frozen cycle and in addition our work though revealed an improvement in pregnancy rate, it was not statistically significant. The same workers Eftekhar et al in their non-randomized clinical trial demonstrated that G-CSF improved implantation and clinical pregnancy rate in infertile women with thin endometrium in frozen-thawed embryo transfer cycles without improving endometrial thickness [24]. The observation of Barad et al also revealed that intrauterine G-CSF administration in fresh IVF cycles with normal endometrial thickness do not alter endometrial thickness, implantation, and clinical pregnancy rates and so did the study by Li et al who also could not prove improved embryo implantation after GCSF infusion [25,26]. The aforementioned researches makes us speculate that G-CSF may

improve pregnancy rates in the presence of poor endometrium and that in normally thickened endometrium there is no added advantage of G-CSF administration.

The background information on the proposed role of G-CSF in female reproduction had led many workers to clinically apply it as a treatment modality for implantation failure, repeated miscarriages and for endometrial expansion. Kim et al found a significant improvement in ongoing pregnancy and implantation rates in women with recurrent IVF failure due to poor endometrial development [27]. They however used the subcutaneous rather than the intrauterine route and multiple injections too.

Various theories and molecular mechanisms have been deciphered and researched on the supposed role of G-CSF in enhancing reproductive outcome. Salmassi et al reported an increased level of serum G-CSF in infertile women who conceived in comparison to women without pregnancy, and concluded that G-CSF have a key role in the pregnancy achievement / maintenance [28]. The work by Rahmati et al. demonstrated that sub fertile women with RIF have a significantly lower level of G-CSF receptors at the maternal-fetal interface, and G-CSF administration would increase the expression of G-CSF receptors and that G-CSF administration locally has the potential to increase the expression of CD16, CD56 which enhance the chance of pregnancy [29]. Rahmati et al have also documented that G-CSF plays a role in modulating genes, involved with adhesion of embryo, cell migration, tissue remodeling and angiogenesis. All of these processes are necessary for embryo implantation and placentation [29]. Taking a cue from all these studies we thus aimed to carry out our work to investigate if it could enhance the pregnancy rate in FET cycles. Even though there was an increase but not of statistical significance. We need to carry out studies on larger number of cycles and in a randomised trial. Some studies have proven subcutaneous route to be superior to intrauterine infusion so that needs to be elucidated further.

Conclusion

The present study found an increase in the clinical pregnancy rate in the G-CSF treated group as compared to the non treated group but this did not reach statistical significance. Thus it would be prudent to carry out larger studies with bigger sample size and a randomised one to elucidate if G-CSF can really improve implantation and pregnancy rates in cycles without any obvious limitations like poor endometrial thickness, recurrent implantation failure or recurrent pregnancy loss.

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