Original Article

Effect of Intrauterine Perfusion of Granulocyte Colony-stimulating Factor on Endometrial parameters and *In Vitro* Fertilization Outcome in Women Undergoing *In Vitro* Fertilization/Intracytoplasmic Sperm Injection Cycles: A Randomized Controlled Trial

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ABSTRACT

Context: Studies have found intrauterine perfusion of granulocyte colony-stimulating factor (G-CSF) to improve endometrial thickness and implantation rates in women undergoing *in vitro* fertilization (IVF). **Aims:** To study the effect of intrauterine perfusion of G-CSF on endometrial parameters and IVF outcomes in patients undergoing fresh embryo transfers. **Settings and Design:** This was a randomized double-blinded placebo-controlled trial conducted at assisted reproduction unit of a tertiary care center. **Subjects and Methods:** One hundred and fifty patients undergoing IVF/intracytoplasmic sperm injection (ICSI) treatment and fresh embryo transfers were randomized to intervention and placebo groups. Patients in the intervention group received intrauterine perfusion of 300 μg (0.5 ml) of G-CSF on the day of ovulation trigger. Patients in placebo group received intrauterine perfusion of 0.5 ml normal saline on the day of ovulation trigger. The primary outcome measure was clinical pregnancy rate. The secondary outcome measures were change in endometrial thickness, volume, and vascularity on the day of embryo transfer; biochemical pregnancy rate, implantation rate, ongoing pregnancy rate, and live birth rate. Statistical analysis was carried out using STATA 12.0 (StataCorp LP, College Station, Texas, USA). **Results:** Endometrial vascularity in the intervention group was significantly higher on the day of embryo transfer compared to the placebo group. Clinical pregnancy rate was 27.6% in the intervention group compared to 18.9% in the placebo group and the difference was not statistically significant (*P* = 0.207). There was no statistically significant difference between biochemical pregnancy rate, implantation rate, ongoing pregnancy rate, live birth rate and endometrial parameters between the two groups. **Conclusions:** Routine use of G-CSF in unselected IVF cycles may not lead to increase in positive IVF outcomes. More trials with larger sample sizes are required before approving or refuting the role of routine G-CSF in increasing IVF success rates. (CTRI/2017/10/010310).

KEYWORDS: *Granulocyte colony-stimulating factor, implantation, in vitro fertilization, pregnancy*

INTRODUCTION

Despite extensive research in the field of assisted reproduction technology (ART), implantation of an embryo is the rate-limiting step in ART cycles. The interaction between a good-quality embryo and

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Access this article online

Quick Response Code:

Website:
www.jhrsonline.org

DOI:
10.4103/jhrs.JHRS_20_18

a receptive endometrium at the time of implantation along with good embryo transfer technique determines the success of ART cycles.[1] A lot of research has been directed toward studying the factors that influence endometrial receptivity and measures to assess and possibly improve endometrial receptivity.

Many noninvasive methods have been studied to assess endometrial receptivity clinically such as ultrasound measurement of endometrial thickness, pattern, volume, and endometrial blood flow. An endometrial thickness of <7 mm has been generally considered as a reliable sign of suboptimal implantation potential.[2,3] Raga *et al.* found implantation rates to be significantly lower in women with endometrial volume <2 ml; no pregnancy was achieved with endometrial volume <1 ml in their study.[4] Uterine artery blood flow has been suggested as a measure of uterine biophysical profile to assess the uterine scoring system for reproduction.[5] None of these parameters have been found to be useful as the sole predictors of endometrial receptivity, and further studies are needed to find the ideal measure of endometrial receptivity.[6]

Various methods have been tried to increase endometrial thickness, its receptivity, and pregnancy rates in ART cycle such as supplementation of estradiol, sildenafil, aspirin, and low-molecular-weight heparin. The safety and efficacy of these drugs in the treatment of infertility have not been well established, and they are not recommended at present.

Successful implantation depends on the interplay between various cytokines secreted by the decidua and the fetal chorionic villi such as colony-stimulating factor, interleukin-1, and leukemia inhibitory factor.[7] Granulocyte colony-stimulating factor (G-CSF) is one such cytokine. Studies by Scarpellini and Sbracia and Santjohanser *et al.* have shown that women treated with subcutaneous G-CSF for recurrent pregnancy losses had a significantly better pregnancy outcome compared to the control group.[8,9] Women with repeated *in vitro* fertilization (IVF) failures showed significantly increased implantation and pregnancy rates with subcutaneous G-CSF administration.[10]

Gleicher *et al.* conducted a pilot study on four women who had previous failed IVF cycles and thin endometrium which was resistant to conventional treatment. All four women benefitted with intrauterine transfusion of G-CSF and demonstrated a significant increase in endometrial thickness. All four women in their study conceived.[11]

Barad *et al.* demonstrated that in general population of women undergoing IVF cycles who did not necessarily have a thin endometrium, intrauterine G-CSF instillation did not lead to significant increase in endometrial thickness or significant improvement in pregnancy rates.[12] Similar results have been shown by Eftekhar *et al.* where no benefit of intrauterine G-CSF instillation was found in women with normal endometrial thickness undergoing IVF-ICSI cycles.[13] However, literature is still sparse on the role of G-CSF in women undergoing IVF treatment. The present study was conducted to evaluate the role of G-CSF in improving endometrial parameters and IVF outcome in an unselected population of women, exploring the benefit of use of G-CSF routinely to improve IVF outcomes in Indian women.

SUBJECTS AND METHODS

This was a randomized controlled trial conducted among 150 women undergoing IVF/ICSI treatment at Assisted Reproductive Technology Centre, Department of Obstetrics and Gynaecology, at a tertiary care hospital. The study was approved by the Institutional Ethics Committee of the Hospital. Women attending IVF clinic were screened for the study. Inclusion criteria were women between 21 and 38 years of age, body mass index (BMI) 18.5–29.9 kg/m², a normal hormonal profile (anti-Müllerian hormone [AMH] >1.5 ng/ml, follicle-stimulating hormone [FSH] <12 mIU/ml on day 3 of menstrual cycle).

Women with a distorted uterine cavity (e.g., fibroid, adenomyoma distorting the endometrial cavity) or history of tubercular endometritis and poor ovarian reserve (AMH <1.5 ng/ml, FSH >12 mIU/ml, antral follicle count [AFC] <6 on day 3 of menstrual cycle) were excluded from the study. Other exclusion criteria were associated with medical problems such as diabetes mellitus, hypertension, heart disease, or drug allergies and women with contraindications to G-CSF administration such as renal disease, sickle cell disease, pneumonia, and malignancies.

Informed written consent was taken from the couple after explaining the detailed plan, purpose, and duration of the study in their own language. After enrolment into the study, patients were randomized into two groups; intervention and control groups, according to the computer-generated randomization table. Individual randomization cards were sealed in numbered opaque envelopes which were accessible to a single person who administered the randomization table and prepared the study materials. Treatment assignment was blinded to patients, doctors administering treatment and performing ultrasounds, and nursing staff. Figure 1 depicts the consort flow diagram for the study.

According to randomization table, 76 patients were randomized to the intervention group and 74 patients
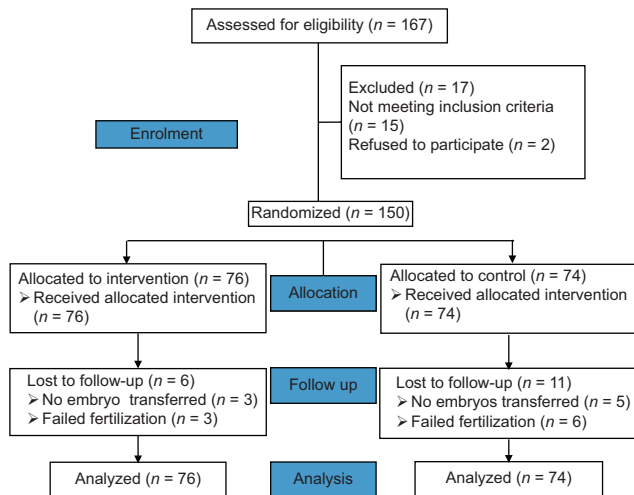
Figure 1: Consort flow diagram

were randomized to the control group. Patients in the intervention group received 300 μg (0.5 ml) of G-CSF (Filgrastim, NUFIL SF™, Biocon Limited, Bangalore) administered by transcervical intrauterine perfusion on the day of ovulation trigger. G-CSF used in the study is available as 0.5 ml single-use prefilled syringe containing 300 μg of Filgrastim (sterile, clear, and colorless preparation). Patients in the control group were administered 0.5 ml of normal saline by transcervical intrauterine perfusion on the day of ovulation trigger. Endometrial thickness, volume, and vascularity were assessed by transvaginal sonography on the day of trigger and at the time of embryo transfer.

The clinician decided on recruitment of subjects for agonist, antagonist, or micro-dose flare agonist protocol depending on the subject's age, BMI, previous IVF response, ovarian reserve, AMH, and FSH levels. Clinician and participants both were blinded about the group allocation.

Luteal phase was supported by micronized progesterone given (100 mg daily) by intramuscular route or micronized progesterone (400 mg twice daily) given by vaginal route for 15 days. Periconceptional folic acid supplementation was given. Patients were followed up for conception by doing urine pregnancy test after 15 days of embryo transfer. If pregnancy was established, progesterone supplementation was continued up to 12 weeks of gestation. The number of gestational sacs and the presence of fetal heart on ultrasound were assessed 28 days after embryo transfer. A repeat ultrasound was done at 12 weeks to note ongoing pregnancy at 12 weeks.

No drug other than those mentioned in the protocol was given to the patient. Any adverse effect was noted. A record of drop outs including premature terminations from the study was maintained.

The primary outcome measure was clinical pregnancy rate. The secondary outcome measures were endometrial thickness, endometrial volume, and endometrial vascularity on the day of embryo transfer, biochemical pregnancy rate, implantation rate, ongoing pregnancy rate, and live birth rate.

Biochemical pregnancy refers to evidence of conception based on the detection of human chorionic gonadotropin in the serum or urine. Clinical pregnancy refers to the evidence of pregnancy by visualization of a gestational sac and embryonic pole with heart beat on the ultrasound. Multiple gestational sacs present in one patient are counted as one clinical pregnancy. Ongoing pregnancy refers to the presence of fetal heart activity confirmed on ultrasound after 12 weeks of pregnancy.

Implantation rate was calculated by dividing the number of gestational sacs (determined by transvaginal ultrasound) present 28 days after embryo transfer by the total number of embryos transferred for each group.

Endometrial thickness was measured on transvaginal ultrasound at its maximum thickness in the longitudinal axis of the uterine body, taking into account the distance between the two basal layers of the anterior and posterior walls at the echogenic interface between endometrium and myometrium.[14]

Three-dimensional ultrasound was done on VOLUSON Scholar-6 GE MACHINE (Model: 083037002028313) using 5–7 MHZ frequency vaginal probe, and endometrial volume measurement was taken using VOCAL imaging program (virtual organ computer-aided analysis) using rotational method by a single trained observer and the data were recorded.

Endometrial color Doppler transvaginal ultrasound was performed, and color mapping of endometrial vascularity was done according to the degree of penetration into the endometrial thickness. The Doppler zones were classified as follows:
- Zone 0 (absent): Absent or negative flow: Only surrounding myometrial vessels seen which do not reach endometrium
- Zone 1 (subendometrial) or peripheral flow: Color signals reach the hyperechogenic outer layer of the endometrium
- Zone 2 (outer hyperechogenic zone): Color mapping occupies the outer half of the endometrial hypoechogenic thickness
- Zone 3 (inner hypoechogenic zone): Vessels reach the endometrial cavity invading the entire endometrial thickness and therefore penetrate all layers of the endometrium[15]

- All the endometrial parameters were measured on the day of ovulation trigger and again on the day of embryo transfer.

RESULTS

A total of 167 patients were screened for the study and 150 patients were recruited according to the inclusion and exclusion criteria. Table 1 shows the baseline characteristics, ovarian reserve, and IVF cycle characteristics of the intervention and control groups.

There was no statistically significant difference between the two groups with respect to IVF cycle characteristics such as IVF protocol followed, gonadotropin dose required, days of stimulation required, number of oocytes retrieved, number of embryos transferred, and cycle cancellation rate. The endometrial thickness measured on the day of trigger was 9.3 ± 1.6 mm for intervention group and 9.4 ± 1.6 mm for control group ($P = 0.660$). Endometrial volume and zone of endometrial vascularity were also comparable between the two groups. Fifty-six (73.7%) patients in the intervention group, and sixty-two (83.8%) patients in the control group had endometrial vascularity in Zone 1 ($P = 0.131$).

Table 2 shows the primary and secondary outcomes in the study.

Table 1: Baseline demographic profile and IVF charactrestics of the two groups			
Characteristic	Intervention (*n*=76)	Control (*n*=74)	*P*
Age (years)*	30.9±3.6	30.0±3.3	0.100
BMI (kg/m²)*	24.3±3.1	23.5±2.6	0.076
Type of infertility†			
Primary	52 (68.4)	56 (75.7)	0.322
Secondary	24 (31.6)	18 (24.3)	
Duration of infertility (years)‡	5 (1–12)	5 (1–13)	0.498
Previous IVF attempts†			
No	70 (92.1)	63 (85.1)	0.178
Yes	6 (7.9)	11 (14.9)	
Reason for IVF†			
Unexplained	17 (22.4)	14 (18.9)	0.333
Tubal factor	31 (40.8)	41 (55.4)	
Male factor	17 (22.4)	12 (16.2)	
PCOS	11 (14.4)	7 (9.5)	
Day 2/3 S. FSH (mIU/ml)*	6.2±1.8	5.9±1.5	0.169
S. AMH (ng/ml)‡	4.0 (2.0–13.5)	3.8 (1.5–9.6)	0.200
AFC*	7.8±2.8	7.7±2.6	0.762
Type of IVF†			
Agonist	43 (56.6)	35 (47.3)	0.427
Antagonist	26 (34.2)	33 (44.6)	
Micro-dose flare agonist	7 (9.2)	6 (8.1)	
Gonadotropin dose required			
rFSH (IU)*	2588.3±863.6	2643.7±1079.4	0.729
HMG (IU)‡	375 (0–3600)	375 (0–5550)	0.418
Day of ovulation trigger			
Estrogen (pg/ml)‡	4207.5 (913–14,778)	4122 (490–11,473)	0.271
Progesterone (ng/ml)‡	1.4 (0–7.0)	1.4 (0.1–7.3)	0.648
Days of stimulation*	11.2±1.6	11.5±1.6	0.372
Number of oocytes retrieved‡	8 (0–24)	8 (0–21)	0.349
Number of embryos transferred‡	2 (0–3)	3 (0–3)	0.310
Cycle cancellation rate†	6 (5.6)	5 (5.4)	0.789
Endometrial thickness on day of trigger (mm)*	9.3±1.6	9.4±1.6	0.660
Endometrial volume on day of trigger (ml)*	5.4±1.7	5.3±1.8	0.716
Endometrial vascularity on day of trigger†			
Zone 1	56 (73.7)	62 (83.8)	0.131
Zone 2/3	20 (26.3)	12 (16.2)	

*Mean±SD, †*n* (%), ‡Median (Minimum value–maximum value). SD=Standard deviation, BMI=Body mass index, IVF=*In vitro* fertilization, PCOS=Polycystic ovarian syndrome, AFC=Antral follicle count, S. AMH=Serum anti-Müllerian hormone, S. FSH=Serum follicle stimulating hormone, HMG=Human menopausal gonadotrophin

Table 2: Primary and secondary outcomes

	Intervention (*n*=76), *n* (%)	Control (*n*=74), *n* (%)	Difference (95% CI)	*P*
Endometrial parameters on day of embryo transfer*				
Endometrial thickness (mm)	11.3±2.0	11.2±1.8	0.3 (−0.49–0.73)	0.697
Endometrial volume (ml)	8.0±2.4	7.5±1.8	0.3 (−0.25–1.12)	0.212
Endometrial vascularity on day of transfer†				
Zone 1	42 (55.2)	59 (79.7)	1.67 (1.24–2.24)	0.001
Zone 2/3	34 (44.8)	15 (20.3)		
Biochemical pregnancy rate†	22/76 (28.9)	15/74 (20.3)	1.24 (0.89–1.72)	0.218
Clinical pregnancy rate†	21/76 (27.6)	14/74 (18.9)	1.25 (0.9–1.74)	0.207
Implantation rate†	27/170 (15.9)	21/174 (12.7)	1.17 (0.89–1.53)	0.396
Ongoing pregnancy rate†	20/76 (26.3)	12/74 (16.2)	1.31 (0.95–1.83)	0.131

*Mean±SD, †*n* (%). SD=Standard deviation, CI=Confidence interval

There was no statistically significant difference between endometrial thickness and endometrial volume between both the groups on the day of embryo transfer. Endometrial vascularity significantly improved on the day of embryo transfer in the intervention group. In intervention group, 42/76 (55.2%) women had Zone 1 endometrial vascularity and 34/76 (44.8%) had Zone 2 or 3 vascularity, while in the control group, 59/74 (79.7%) women had Zone 1 vascularity while 15/74 (20.3%) had Zone 2 or 3 endometrial vascularity (*P* = 0.001).

Although the absolute number of biochemical pregnancies, clinical pregnancies, and ongoing pregnancies and live births were more in the intervention group, the difference was no statistically significant.

DISCUSSION

The present study was done to evaluate the role of intrauterine perfusion of G-CSF on endometrial parameters and IVF outcomes. The study failed to show any benefit of routine use of intrauterine G-CSF perfusion on clinical pregnancy rate and live birth rate in women undergoing IVF-ICSI cycles.

Gleicher *et al.* did a pilot study on four women with previous failed IVF cycles and thin endometrium resistant to conventional treatment. Intrauterine instillation of G-CSF led to significant increase in endometrial thickness and all four patients in the study conceived.[11] Kunicki *et al.* found similar results in their study on 37 women with thin endometrium undergoing IVF.[18]

Li *et al.* failed to demonstrate in their study on women with thin endometrium undergoing IVF that G-CSF administration led to increased implantation or pregnancy rates.[19] Another clinical trial on women with thin endometrium found similar results.[20]

Barad *et al.* studied the benefit of intrauterine G-CSF instillation in women with normal endometrium undergoing IVF and found no significant increase in endometrial thickness or improvement in pregnancy rates as compared to controls. However, women in their study had increased age, slightly raised FSH, and lower AMH values, limiting the application of their study to general population undergoing IVF.[12]

As compared to mean age in study by Barad *et al.*, women in the present study were more representative of population of women undergoing IVF with respect to age, ovarian reserve, and previous failed IVF records.[12]

The mean endometrial thickness on the day of embryo transfer was not statistically significant between control and intervention groups in the present study, similar to the findings in the study by Barad *et al.*[12] Endometrial thickness increased by 1.75 (0.3–8.1) mm in the intervention group and 1.6 (0.2–5.6) mm in the control group from the day of ovulation trigger to the day of embryo transfer (*P* = 0.368).

Raga *et al.* found in their study that patients with endometrial volume <2 ml had lower pregnancy and implantation rates compared to the groups of women with endometrial volume 2–4 ml and >4 ml (*P* < 0.05).[4] Endometrial volume may therefore be used as a predictor of endometrial receptivity. In the present study, intrauterine instillation of G-CSF did not lead to statistically significant difference in endometrial volume on the day of embryo transfer between the intervention and control groups.

In the present study, women in the intervention group who received G-CSF were more likely to have endometrial vascularity in Zone 2 or 3 than women in the control group. Kupesic *et al.* reported a low resistance index and high flow index in endometrial Doppler indices to be predictive of a more favorable endometrial milieu for implantation.[16] Singh *et al.* have also found endometrial vascularity to be

useful in predicting successful implantation in IVF cycles.[17]

Women who received G-CSF in the study were 25% more likely to have a clinical pregnancy compared to the placebo group although this value was not statistically significant (*P* = 0.207, relative risk 1.25, confidence interval: 0.9, 1.74). These findings are consistent with the studies by Barad *et al*. and Eftekhar *et al*. There was no statistically significant difference between biochemical pregnancy rate, implantation rate, and ongoing pregnancy rates between the two groups, which is consistent with the findings of studies by Barad *et al*. and Eftekhar *et al*.[12,13]

This study failed to demonstrate the beneficial effect of G-CSF in improving pregnancy outcomes, endometrial thickness, and endometrial volume in regular IVF cycles. There was a significant improvement in endometrial vascularity with the use of G-CSF, but this did not translate into a successful pregnancy outcome.

The control group in our study had clinical pregnancy rate of which is quite low as compared to the general IVF success rate. Intrauterine instillation of saline may be thought as possible cause of low implantation. There was lack of a third group with no intervention which could have explained this low implantation rate in the saline infusion group. Furthermore, the control group had more numbers of patients with previous failed cycles though the difference was not statistically significant.

More number of randomized controlled trials with larger sample sizes exploring dosage, timing, and route of G-CSF instillation are required before approving or refuting the role of routine G-CSF administration in increasing success rate of IVF cycles.

CONCLUSION

The present study does not support the routine use of intrauterine perfusion of G-CSF in women undergoing IVF cycles. More prospective large sample size trials are required to approve or refute its routine use to improve success rate of IVF cycles.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

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