

RESEARCH ARTICLE

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

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ABSTRACT

Introduction: In spite of significant advances in the field of reproductive medicine, repeated implantation failure (RIF) is a challenging and extremely disappointing problem. The success of *in vitro* fertilization and embryo transfer (IVF-ET) cycles depends mainly on uterine receptivity and embryo quality. Successful evaluation of endometrial receptivity conducive to embryo implantation continues to be a challenge in assisted reproductive technology (ART). Several researcher groups have reported the successful use of granulocyte-colony stimulating factor (G-CSF) during IVF cycles in terms of achieving higher clinical pregnancy rates on account of increased endometrial thickness. Women who fail to achieve adequate endometrial thickness despite conventional treatment with high dose estrogen or those with a history of repeated implantation failures in spite of normal endometrial thickness often do not achieve pregnancy and resort to gestational surrogacy. A new therapeutic approach to achieve successful pregnancy in such patients would be very desirable.

Study objectives: To assess the efficacy of a single dose of intrauterine G-CSF on endometrial thickness, implantation and clinical pregnancy rates in women who either had a thin endometrium after estrogen priming (<8 mm) or a history of repeated implantation failures at IVF, undergoing embryo transfer after 10 days of priming with oral estradiol and vaginal sildenafil.

Materials and methods: Two hundred and thirty-one women (between 24 and 46 years of age) undergoing IVF-ET were recruited for the study. All cases were prospectively studied at the Mumbai Fertility Clinic and IVF Center (a subdivision of Kamala Polyclinic and Nursing Home) over a period of 6 months from January to June 2014, after their written informed consent. Subgroup I consisted of 117 patients who had a persistently thin endometrium (<8 mm) in spite of high dose oral estradiol valerate and vaginal sildenafil priming. Subgroup II consisted of 114 patients who had a history of repeated (two or more) implantation failures (RIF) at IVF-ET cycles despite adequate endometrium (≥ 8 mm). All were infused with a single dose of G-CSF (300 mcg) in the uterine cavity after 10 days of priming with oral estradiol valerate and vaginal sildenafil citrate. Endometrial thickness was reassessed 4 days after G-CSF instillation. This was followed by administration of intramuscular progesterone in oil (100 mg) daily with embryo transfer on day 5 of progesterone

for all patients. All embryo transfers for patients undergoing oocyte donation or embryo donation were done at the 4-cell stage on day 2. All Frozen embryo transfers (FET) of vitrified embryos were at 8 cell stage. Estimation of serum beta hCG was at 14 days post-embryo transfer for all patients. Successful implantation and net clinical pregnancy rate was confirmed based on appearance of gestational sac on sonogram after 10 days and observation of fetal cardiac activity after 20 days of positive β -hCG results.

Results: Out of total 231 patients recruited in the study, 95% patients from subgroup I (n = 111) and 94% patients from subgroup II (n = 107) showed mean increase in endometrial thickness by at least 2.5 mm within 4 days of G-CSF single dose instillation. A total of 218 patients from both subgroups underwent S. β -hCG estimation 14 days post IVF-ET. Out of 103 β -hCG positive patients, 83 showed net clinical pregnancy (fetal cardiac activity present) giving a net pregnancy rate of 38.07% for the whole study group with 37% in the subgroup with thin endometrium (<8 mm) and 39.25% in the subgroup with adequate (≥ 8 mm) endometrium with history of two or more failed implantation at previous IVF-ET cycles. There were no adverse events for the whole study population.

Conclusion: There can be a strong possibility with a single dose of 300 mcg intrauterine infusion of G-CSF to achieve significant increase in the endometrial thickness with higher successful pregnancy rate among infertile women undergoing IVF-ET cycles with a history of a persistently thin endometrium or repeated implantation failures (rather difficult to treat patients). G-CSF could be a valuable tool to consider before advising the option of surrogacy. In the absence of a control group, our conclusions warrant conduct of further studies.

Keywords: Endometrial thickness, Granulocyte colony stimulating factor, *In vitro* fertilization, Embryo transfer, β -hCG.

How to cite this article: 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.

Source of support: Nil

Conflict of interest: None

Date of Received: 02-12-14

Date of Acceptance: 08-12-14

Date of Publication: September 2014

INTRODUCTION

In spite of significant advances in the field of reproductive medicine, repeated implantation failure (RIF) is a challenging and extremely disappointing problem faced

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by the clinicians and the couples alike. The success of *in vitro* fertilization and embryo transfer (IVF-ET) cycles depends mainly on uterine receptivity and embryo quality.¹ Evaluation of endometrial receptivity for implantation continues to be a challenge in assisted reproductive technology (ART). Many studies have implicated endometrial thickness and pattern as prognostic parameters for successful outcomes in IVF-ET. The problem of chronically thin endometrium resistant to standard treatments in patients undergoing IVF is of considerable importance as such thin endometrium is widely considered suboptimal for transfer and associated with reduced pregnancy chances.²⁻¹⁰ Several researcher groups have reported the successful use of granulocyte-colony stimulating factor (G-CSF) during IVF cycles in terms of increasing the endometrial thickness and thereby achieving higher clinical pregnancy rates.

Granulocyte-colony stimulating factor is a cytokine that stimulates neutrophilic granulocyte proliferation and differentiation. It is indicated for the treatment of neutropenia owing to cancer chemotherapy. A patent application of the year 2009 in United States has already claimed benefit from G-CSF treatment in cases of implantation failure and repeated pregnancy loss, suggesting that G-CSF can affect the endometrium. Preliminary studies have demonstrated that G-CSF stimulates neutrophilic granulocyte proliferation and differentiation, acts on macrophages of decidual cells, and finally affects the implantation.^{11,12} Moreover, some immune effects of G-CSF lead to recruitment of dendritic cells, promoting Th-2 cytokine secretion, activating T regulatory cells, and also stimulation of various proangiogenic effects.^{12,13} The receptor for G-CSF is expressed by the trophoblastic cells and by human luteinized granulosa cells.^{14,15} It is also stated that G-CSF prevents repeated miscarriages and implantation failures.^{16,17} Gleicher et al presented two clinical studies with limited number of participants regarding the usefulness of G-CSF treatment in endometrial expansion in women who had previously cancelled cycles because of the unresponsive endometrium.^{18,19}

Women who fail to achieve adequate endometrial thickness despite receiving different treatments or those with the history of failed implantation at repeat IVF cycles irrespective of their endometrial thickness often do not undergo embryo transfer and resort to gestational surrogacy. A new therapeutic approach to achieve successful pregnancy in such patients would be very desirable. Taking into account all these data, the aim of the present study was to examine the effects of G-CSF on endometrial thickness and clinical pregnancy rates in a subsection of poor prognosis women undergoing *in vitro* fertilization (IVF).

STUDY OBJECTIVE

To assess the efficacy of a single dose of intrauterine G-CSF on endometrial thickness, implantation and clinical pregnancy rates in women who either had a thin endometrium after estrogen priming (<8 mm) or a history of repeated implantation failures at IVF, undergoing embryo transfer after 10 days of priming with oral estradiol and vaginal sildenafil.

MATERIALS AND METHODS

The study was conducted at a private infertility clinic in Mumbai, India. A total of 231 patients participated in the study after giving their informed consent. The mean age for the study group was 33.48 ± 3.79 years with the range being 24 to 46 years. The subjects were divided in two subgroups. Subgroup I comprised of 117 patients who had an endometrial thickness of <8 mm despite priming with high dose estradiol valerate and vaginal sildenafil while subgroup II (n = 114) comprised of those with two or more failed implantations with IVF-ET despite an endometrial thickness of ≥ 8 mm at the time of G-CSF instillation (n = 114). Contraindications for G-CSF treatment like sickle cell disease, chronic neutropenia, known past or present malignancy, renal insufficiency, upper respiratory infection, pneumonia and congenital fructose intolerance, Asherman's syndrome, fibroids, and polyps in diagnostic hysteroscopy were ruled out to confirm eligibility of participation. Patients undergoing embryo transfer in fresh stimulated cycles were excluded from the study to avoid variations in implantation rates due to variations in embryo quality or factors related to ovarian stimulation, such as a premature rise in progesterone and endometrial advancement or high estradiol levels resulting in apoptosis of endometrial glands.

All eligible patients in both groups underwent 10 days priming with oral estradiol valerate (6-8 mg/day) and vaginal sildenafil citrate (25 mg TID) which was initiated from the 4th day of the menstrual cycle. After recording baseline endometrial thickness, all patients underwent a single intrauterine instillation of injection G-CSF 300 mcg. The instillation of G-CSF was done with an IUI catheter (from Surgimedik, India) which was introduced beyond the internal os almost up to the fundus. Endometrial thickness was reassessed for all 231 patients after 4 days of G-CSF instillation. During the study, all patients received progesterone either as 100 mg daily IM injection in oil or as 8% topical vaginal gel twice daily beginning 4 days after G-CSF instillation and continued until performing the pregnancy test (S β -hCG) 14 days after embryo transfer. Progesterone administration was further continued for all β -hCG positive patients for

20 more days until cardiac activity was visualized. Embryo transfer (average 1-3 embryos) was done in both groups on the 5th day of progesterone initiation using frozen own embryos (previously cryo-preserved vitrified embryos) or fresh donor eggs or donor embryos. During the IVF cycles, some of the patients received oocyte donation with IVF using husband sperms or with intracytoplasmic sperm injection containing husband sperms. All thawed embryos were eight-cell stage with vitrification done at four-cell stage. All oocyte and embryo donation cycles had day 2 embryo transfer at four-cell stage. A maximum of three embryos were transferred to each patient while average being 1 to 3 embryos. All donor egg and donor embryo cycles had day 2 transfers at the four-cell stage. All vitrification transfers (FET) were at eight-cell stage. Patients showing positive β -hCG results (14 days post-embryo transfer) were checked for appearance of gestational sac through transvaginal ultrasonography (USG) after 10 days followed by monitoring of fetal cardiac activity after another 10 days, i.e. after 20 days of pregnancy detection (based on positive β -hCG results).

STUDY END-POINTS

1. Mean change in endometrial thickness against baseline measured in transvaginal ultrasonography after 4 days of G-CSF instillation.
2. Successful implantation and clinical pregnancy rate after embryo transfer based on serum β -hCG estimation, evidence of a gestational sac on USG and monitoring of fetal cardiac activity.

STATISTICAL ANALYSIS

Continuous variables were presented as mean \pm SD; categorical variables were presented in percentage. Differences between dependent variables (before and after) were checked by paired t-test. $p < 0.05$ was considered statistically significant. The statistical package SAS (data analysis software system), version 9.3 was used for data analysis. Successful implantation and clinical pregnancy rate was analyzed for both subgroups using Chi-square test.

RESULTS

Efficacy Assessment

In 231 subjects at the time of instillation of G-CSF, mean baseline endometrial thickness recorded was 7.98 ± 1.30 mm. 95% patients from subgroup I ($n = 111$) and 94% patients from subgroup II ($n = 107$) showed mean increase in endometrial thickness to 10.97 ± 1.23 mm (Fig. 1) within 4 days of G-CSF single dose instillation. The mean change in endometrial thickness observed

was 2.99 ± 0.86 mm ($p < 0.0001$). Most patients showed an increase in endometrial thickness by at least 2.5 mm. Almost 50% patients had an increase of 3 mm or more in endometrial thickness.

When we divided the whole group into two subgroups according to baseline endometrial thickness and history of two or more failed IVF cycles, we observed mean increase in endometrial thickness up to 3.24 ± 0.86 mm in group with baseline thickness < 8 mm and up to 2.73 ± 0.78 mm in the group having a history of two or more failed implantations at IVF but normal endometrial thickness (≥ 8 mm) ($p < 0.0001$) (Fig. 2).

There was 0.51 mm difference observed between the two subgroups in respect to the endometrial thickness both before and after G-CSF instillation ($p < 0.0001$). Out of a total of 231 patients, 13 patients did not undergo embryo transfer as there was no increase in the endometrial thickness with G-CSF. For the remaining 218 patients, 103 (47.25%) showed positive serum beta hCG, whereas 115 (52.75%) showed negative serum beta hCG (Fig. 3).

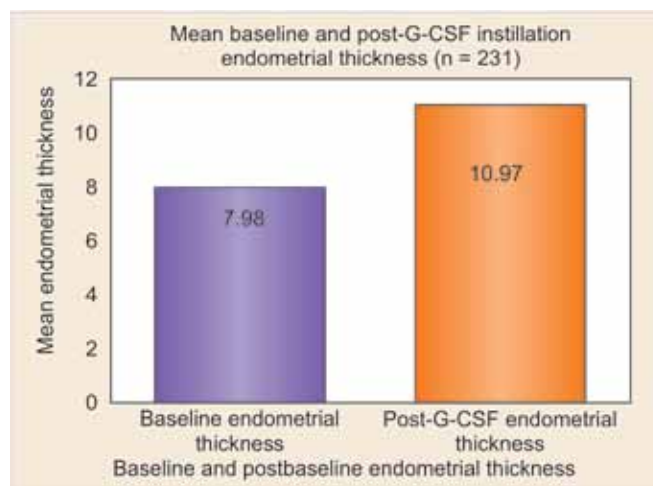


Fig. 1: Mean baseline endometrial thickness before and after granulocyte colony-stimulating factor instillation

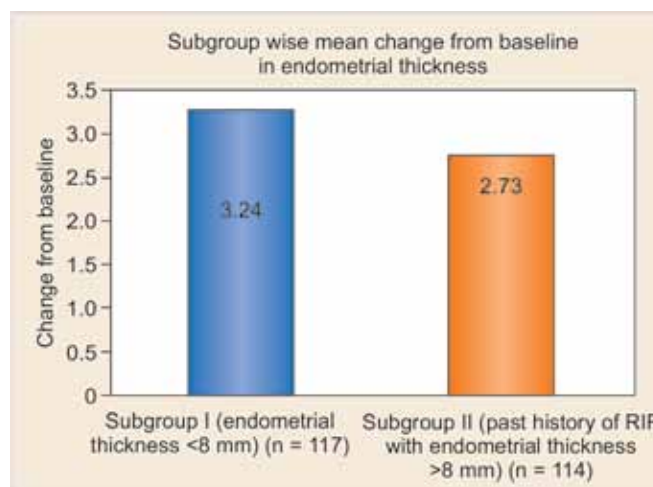


Fig. 2: Mean change in baseline endometrial thickness after granulocyte colony-stimulating factor instillation

Out of 218 patients, 111 belonged to subgroup I with endometrial thickness <8 mm while the remaining 107 were of subgroup II with endometrial thickness \geq 8 mm and with history of RIF at previous IVF-ET. For subgroup I, 56 (50.45%) patients out of 111 showed positive serum β -hCG estimation while for subgroup II, 47 (43.93%) patients out of 107 showed positive serum β -hCG estimation (Fig. 4).

Total 103 patients (out of 218 evaluable patients) with positive β -hCG in both the groups were followed for ongoing clinical pregnancy rate based on appearance of gestational sac (10 days after β -hCG estimation) and monitoring of fetal cardiac activity (20 days after β -hCG estimation). Out of 103 positive β -hCG patients, 20 pregnancies (15 from thin endometrium subgroup and 5 from subgroup with RIF) were lost owing to missed abortion (gestational sac seen but no fetal cardiac activity was evident) or biochemical pregnancy (positive serum β -hCG but low levels, inadequate doubling of levels at 48 hours unlike a normal pregnancy—no gestational sac seen at any stage) or on account of ectopic

pregnancy. The remaining 83 patients (41 from thin endometrium subgroup and 42 from the subgroup with RIF) had successful implantation and ongoing clinical pregnancy based on appearance of fetal cardiac activity. This gave a net successful clinical pregnancy rate of 38.07% (Fig. 5) for the whole study group (n = 218), 37% in subgroup I with thin endometrium (<8 mm) and 39.25% in subgroup II with adequate (\geq 8 mm) endometrium and history of RIF (Fig. 6). Comparison between the two subgroups was found statistically insignificant ($p = 0.8272$).

There was no adverse event reported with intrauterine instillation of G-CSF in our study. However, it was demonstrated before that the treatment with subcutaneous administration of G-CSF could lead to bone pain, general fatigue, headaches, insomnia, anorexia, nausea, and/or vomiting.²⁰ Additionally, dyspnea, chest pain, hypoxemia, diaphoresis, anaphylaxis, syncope and flushing have also been reported for subcutaneous G-CSF upon review of the existing literature.³⁰

DISCUSSION

It has been demonstrated before that <1% of women worldwide have thin endometrium.^{21,22} A thin and unresponsive endometrium is still a major and unresolved clinical problem often leading to repeated implantation failures at IVF. There are inconclusive data regarding the thickness of so-called thin endometrium with various investigators proposing 7, 8 and 9 mm as optimal for clinical pregnancy.²¹⁻²⁵ Several methods have been proposed to increase the thickness of persistently thin endometrium in women undergoing IVF. These therapies included tocopherol, pentoxifylline, low-dose aspirin, sildenafil citrate and estradiol administration.^{24,26,27}

On the other side, in some investigations, there was no correlation between endometrial thickness and IVF

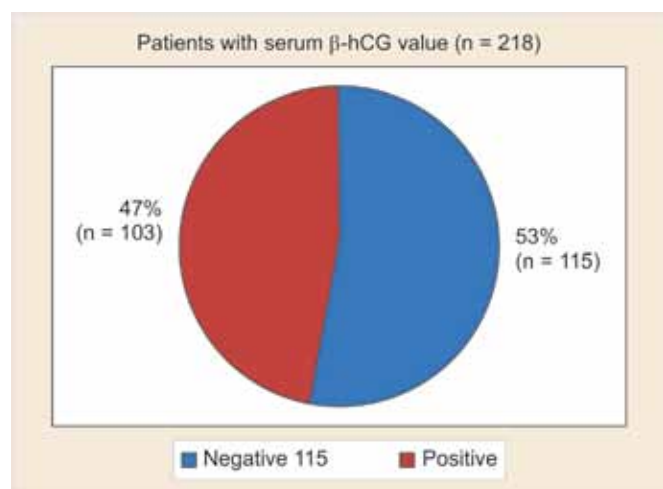


Fig. 3: Serum beta-human chorionic gonadotropin estimation after granulocyte colony-stimulating factor instillation

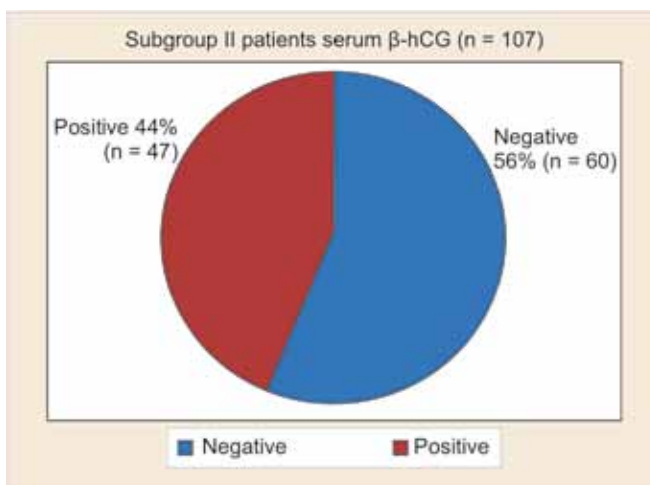
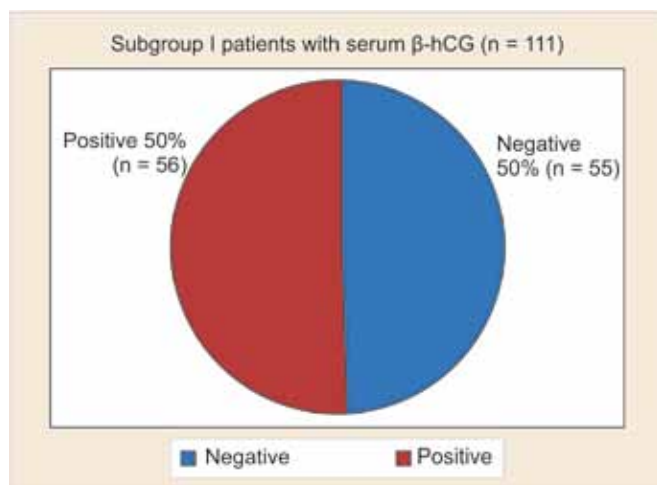


Fig. 4: Percentage of beta-human chorionic gonadotropin positive patients in each subgroup after undergoing *in vitro* fertilization and embryo transfer

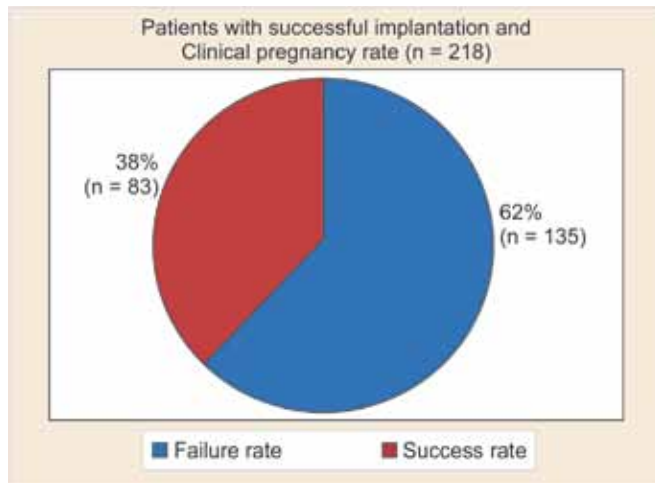


Fig. 5: Net clinical pregnancy rate for all undergoing *in vitro* fertilization and embryo transfer after granulocyte colony-stimulating factor instillation

outcome and endometrium thickness.^{28,29} In the pilot study of Gleicher et al, the authors reported the role of G-CSF on increasing endometrial thickness in women with an unresponsive endometrium.¹⁸ In this case report, the data of four patients infused with G-CSF into the uterus were demonstrated. All these patients finally conceived. Two years later, the same group of authors described 21 patients with a persistently thin endometrium infused with G-CSF. As a result, 19.1% ongoing clinical pregnancy rate was observed. The findings of Gleicher et al provided evidence that G-CSF could be a promising agent in the treatment of women with thin unresponsive endometrium.¹⁹

In our study, we found that the endometrium increased significantly ($p < 0.0001$) after infusion of G-CSF in most examined women ($n = 231$) who were divided in two subgroups according to their endometrial thickness and history of IVF cycles. The increase in endometrium thickness was greater in the subgroup of women with an endometrial thickness of < 8 mm and the difference between the two subgroups was statistically significant. A total 218 women underwent embryo transfer after G-CSF infusion. Serum β -hCG estimation was positive for 103 (47.25%) patients with ongoing clinical pregnancy in 83 patients demonstrating success rate of 38.07% for the whole study group ($n = 218$) which was much higher than the previously reported pregnancy rates in the literature. The previously reported pregnancy rates were in fresh stimulated cycles. Our study deals with cryofrozen embryos and donor eggs and embryos. The success rate was almost 37% in the subgroup with thin endometrium (< 8 mm) and 39.25% in the subgroup with adequate (≥ 8 mm) endometrium but RIF. Of course, most of the reported studies used G-CSF during stimulation cycles (with G-CSF being instilled on the day of hCG and sometimes repeated after 48 hours) unlike our

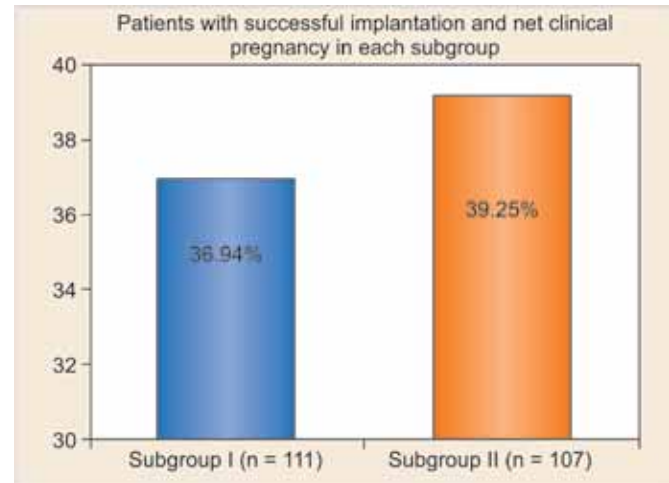


Fig. 6: Net clinical pregnancy rate in each subgroup undergoing *in vitro* fertilization and embryo transfer after granulocyte colony-stimulating factor instillation

study which included only recipient cycles (FET/donor egg/donor embryo). This was intentionally done to remove the study bias of variation in results owing to oocyte or embryo quality issues.

Our study is not without limitations. Firstly, we did not have a control group which received placebo. Thus, the changes of endometrial thickness could be observed only before and after infusion and between two subgroups of women based on their endometrial thickness and history of unsuccessful past IVF cycles. It is however noteworthy that the group with previous implantation failures had failed with the same treatment modality. For example, a patient with two failed oocyte donation cycles was given only donor eggs even in this cycle. In no patient was the treatment modality different. For example, if a patient has failed with her own eggs two times and then appears in this study group for egg donation she would obviously have a higher success rate. We think that, taking into account all previous failures, the G-CSF effect could play the main role in our study. Therefore, the final assessment on how G-CSF affects the endometrium thickness remains open until prospectively controlled studies would be performed. There is also a question on how to properly counsel the patients who did not conceive. We did not come across any adverse events during intrauterine G-CSF infusion that are otherwise reported with subcutaneous G-CSF like bone pain, general fatigue, headaches, insomnia, anorexia, nausea, vomiting, dyspnea, chest pain, hypoxemia, diaphoresis, anaphylaxis, syncope and flushing.^{20,30}

CONCLUSION

In summary, we showed that in women who had thin endometrium or two or more failures in the previous IVF cycles despite having adequate endometrium; the infu-

sion of G-CSF not only increases the endometrial thickness but also leads to higher ongoing clinical pregnancy rates. Additionally, the increase in endometrial thickness was observed within 4 days of G-CSF administration. Because of no control group, our conclusions are limited. We should also remember that the threshold is different in many other studies; thus, clinical pregnancy was observed even in women with endometrium <4 mm.³¹ We think that, despite the obvious limitations, our data from almost 238 patients is important for doctors and couples seeking fertility assistance. G-CSF could be a valuable tool to help increase pregnancy rates among infertile women undergoing IVF-ET cycles irrespective of their endometrial thickness or past treatment failures (rather difficult to treat patients). It could be an extremely important addition in the armamentarium of antifertility agents before advocating the option of surrogacy to infertile women. However, further studies are needed in this field.

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