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Original article

Granulocyte-colony stimulating factor may improve pregnancy outcome in patients with history of unexplained recurrent implantation failure: An RCT

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Abstract

Background: Family of colony-stimulating factors (CSF) have an essential role on early cross talk between embryo and uterine endometrium.

Objective: The aim of this study was to evaluate the effects of the single dose of Granulocyte-CSF (G-CSF) injection on clinical outcome of assisted reproductive technology cycle in patients with repeated implantation failures.

Materials and Methods: This randomized control trial study was performed on 52 infertile women who referred to the clinic with the history of more than three previous In vitro fertilization/Intracytoplasmic sperm injection-embryo transfer failures. All patients were stimulated with standard long protocol. All embryos were transferred on day five in blastocyst stage in both groups. The treated group received 300 µg (0.5 ml) recombinant human G-CSF subcutaneously which was injected 30 min before blastocyst embryo transfer.

Results: There was not statistically significant differences in abortion rate in G-CSF and control group (p=0.09). G-CSF treated group showed higher clinical pregnancy rate in comparison with control group (56.2% vs. 40.0%) but it was not statistically significant (p=0.09). Although live birth rate in G-CSF group was higher than control group (53.1% vs. 35.0%) but there wasn't statistically significant difference in the overall live birth rate between the two groups (p=0.10). G-CSF group had a twin pregnancies while in control group there was no twin pregnancy.

Conclusion: Our result demonstrates the possibility that pregnancy outcome is better in women with repeated unexplained In vitro fertilization failure who are treated with G-CSF.

Key words: *Granulocyte colony-stimulating factor, Embryo implantation, Pregnancy rates, Assisted reproductive technology, Randomized controlled trial.*

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Introduction

Repeated implantation failure (RIF) defined as the case whereby the transferred embryos fail to implant after several In vitro fertilization (IVF) attempts which causes deep impact on the quality of life, and financial burden (1, 2). Endometrial receptivity is the limiting factor for implantation and success of IVF programs (2). Granulocyte-colony stimulating factors (G-CSF) is a hematopoietic lineage-specific cytokine and known for its specific effects on the activation of intracellular signaling pathways that are associated with the cell proliferation, differentiation, and stimulation of hematopoietic cells of the neutrophilic granulocyte lineage (3, 4). Several non-hematopoietic cell types, such as reproductive tissue cells, also have shown to produce G-CSF (5- 7).

During the maturation of the pre-ovulatory follicle, G-CSF receptor expression increases; which also takes place in human endometrium and luteinized granulosa cells. G-CSF receptors also exist on the trophoblast. The highest G-CSF receptor expression occurs in the first trimester (8). G-CSF effect on recruitment of type 2 T helper cytokine secretion, activation of T regulatory cells, modulation of uterine natural killer cells cytotoxicity and endometrium angiogenesis as

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a result has an essential role on early cross talk between embryo and uterine endometrium (3, 7-9). Studies showed that elevated G-CSF concentrations on follicular fluid increased implantation rate and can improve IVF outcome (10-15).

Due to our best knowledge, this study evaluates the effects of the single dose G-CSF injection in a group of patients with unexplained RIFs in whom embryo transfer has been done in blastocyst stage.

Materials and methods

Patients' selection

This randomized control trial study was performed on 132 infertile women (22-44 yr old) at Genetics and In Vitro Assisted Reproductive (GIVAR) Infertility Center in Erfan Hospital, Tehran, Iran, from May 2010 to October 2015 who referred to the clinic with the history of more than two previous IVF/Intracytoplasmic sperm injection-embryo transfer (ET) failures despite transfer of at least two good-quality embryos in each attempt.

Patients were divided into G-CSF and control group by random allocation software base on file number and one by one selection. After taking history and physical exam; hysterosalpingogram, and routine hematological, biochemical and hormonal tests, Karyotype, semen analysis, and also flow-cytometry, autoantibodies profile and thrombophilia profile have accomplished in all women. Excluding criteria was including abnormalities in hysterosalpingogram, thrombophilia, immunological and, genetics problems, and also severe male factor infertility. According to Würfel study (6) the treated group received a single dose of subcutaneous G-CSF and control group received routine procedure (Figure 1).

Treatment protocols

All patients were stimulated with standard long protocol, gonadotrophin releasing hormone agonist (Superfact, Aventis Pharma, Germany) from day 21 of the cycle proceeding the stimulation cycle. Then from the second to the third day of the stimulation cycle, patients received follicle-stimulating hormone (Gonal-F, Merck Serono, Germany) 150-300 units daily. When at least three follicles had a diameter >18 mm, 10,000 IU unit of Human chorionic gonadotropin was administered. After 34-36 hr, puncture of ovaries was done. All embryos were transferred on day five in blastocyst stage in both groups. Cyclogest (Actavis, UK) 400 mg twice daily were given to all patients as luteal phase support. The main outcome measured was pregnancy rate per ET procedure. Secondary outcomes were abortion rate and multifetal pregnancy.

The treated group received 300 µg (0.5 ml) recombinant human G-CSF (300 µg, Zahravi Co., Tehran, Iran) subcutaneously injected 30 min before blastocyst embryo transfer. Pregnancy outcomes like clinical pregnancy and abortion rate were assessed. Clinical pregnancy was defined as the presence of gestational sac with fetal heart beat by ultrasound 4 wk following the ET. Embryo quality was assessed using embryo morphology and divided to 3 categories (A, B, and C). In A category, all blastomeres were symmetric and without fragmentation; In B category, blastomeres were symmetric with 10-50% fragmentation; and In C category, blastomeres were asymmetric with ≥50% fragmentation. Embryo quality was evaluated by an embryologist at the inverted microscope.

Ethical consideration

All of the patients signed an informed consent that allows review of their medical records for research purposes, as long as the patient's anonymity and confidentiality of their medical record are maintained. Investigations and the trial have been approved by Ethical Committee of Shahid Beheshti University of Medical Sciences (SBMU.REC.1393.144).

Statistical analysis

After recording and collecting the data, statistical analysis was performed using the

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Statistical Package for the Social Sciences (SPSS, version 16.0, SPSS Inc, Chicago, Illinois, USA). Patient characteristics such as age, infertility duration, day of female cycle that embryo was transferred and number of embryo transfer and number of good quality embryo transferred was evaluated with t-test or Chi-square. Continuous variables were presented as mean±SD and pregnancy outcome in two groups was analyzed assessed by Chi-square and t-test. The statistical significances considered as $p<0.05$.

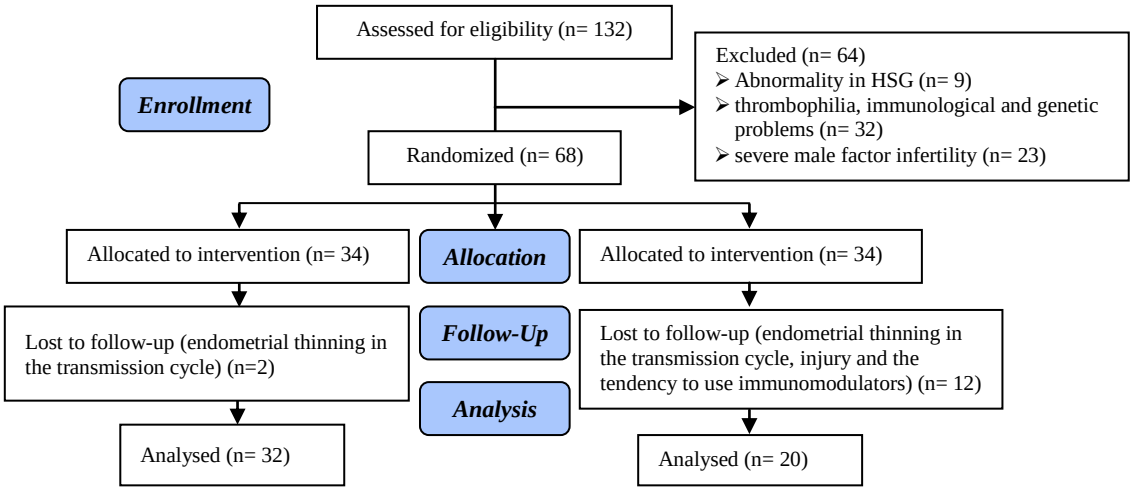

Figure 1. Consort flow diagram. HSG: hysterosalpingogram

Results

From 132 patients referred with RIF, a total of 68 women who have inclusion criteria were randomly assigned to undergo treatment with G-CSF as G-CSF group or the usual treatment as control group (34 patients in G-CSF group and 34 patients in control group). Two patients in the G-CSF group and twelve in the control group was excluded from study for various reasons including endometrial thinning in the transmission cycle and the tendency to use immunomodulators. Finally 52 patients (32 patients in G-CSF group and 20 patients in control group) were elected for the study as unexplained repeated IVF failure. Baseline characteristics of study group are shown in table I. Although the live birth rate in G-CSF group was higher than control group but there was positive but not statistically significant difference in the overall live birth rate between the two groups (p=0.10, t-test).

There were no significant differences in age (p=0.21), infertility duration (p=0.18), duration of the cycle (p=0.10) and number of embryos between groups (p=0.80). Also there was not any differences in the quality of transferred embryo between groups (p=0.53). G-CSF treated group showed higher clinical pregnancy rate, 18 out of 32 (56.2%) in comparison with control group 8 out of 20 (40.0%) (Table I) but this difference was not statistically significant (p=0.09, Chi-square). Also in the G-CSF treated group, 2 abortions out of 18 pregnancies (6.2%) occurred in comparison with 1 abortion out of 8 pregnancies (5%) in the control group (Table I).

The findings showed that there was not statistically significant differences in abortion rate in the group that received G-CSF and control (p=0.09, Chi-square).In G-CSF group we have a twin pregnancy and in control group there was no twin pregnancy. In this study take home baby rate was 53.1% in G-CSF (17 of 32), and 35.0% in control (7 of 20) participants (Figure 2).

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Table I. Comparison of demographic and clinical characteristics of participants.

Variable	G-CSF group (n= 32)	Control group (n= 20)	p-value
Age (yr) *	34.53 ± 5.50	34.05 ± 6.5	0.21
Duration of infertility (yr) *	5.60 ± 3.29	6.00 ± 2.75	0.18
Cycle duration (day) *	15.84 ± 1.58	15.55 ± 2.03	0.10
No. of embryo transferred *	3.31 ± 0.85	3.20 ± 0.95	0.80
No. of good quality embryos (A+B) *	2.93 ± 1.01	3.15 ± 1.03	0.53
Pregnancy rate **	18 (56.2)	8 (40)	0.09
Abortion rate **	2 (6.5)	1 (5)	0.09

*Data presented as mean±SD. Student T-test
**Data presented as n (%). Chi square teast
p-value≤ 0.05 was considered statistically significant.

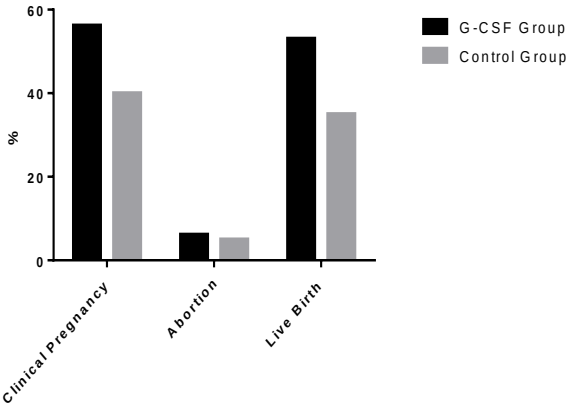

Figure 2. Pregnancy outcome in the G-CSF group compared to control. Note: data present as percentage.

Discussion

Vital embryo and receptive endometrium as well as effective embryo-endometrium dialogue is essential in successful implantation and establishment of pregnancy. However what exactly make the endometrium receptive is not fully understood yet. Endometrial remodeling at implantation window and switching local immunity from the adaptive (type 1 T helper) to the innate (type 2 T helper) type is crucial for implantation (16). Balanced local immune system is required during the implantation window to enable the embryo not only to attach but also to regulate the invasion phase. G-CSF stimulates type 2 T helper cytokine secretion and activation of T regulatory cells so it could be the effective treatment in patient with history of implantation failure (3). In the present study, the pregnancy outcome improved in women used single dose of G-CSF, although this difference was not statistically significant compared with women in the control group. Eftekhar and colleagues showed that intrauterine infusion of G-CSF in infertile women with the history of implantation failure is an effective treatment and can improve the pregnancy outcome (17).

Wurfel *et al* concluded that the use of G-CSF is an extremely promising additional method of treatment in case of implantation failure (18). The improved pregnancy rate might be due to an increase in regulatory T cells and dendritic cells and appeared to influence endometrial expression of genes crucial for implantation process, including endometrial vascular remodeling, local immune modulation and cellular adhesion pathways (3). Our result was different from Wurfel study, in which several dose of G-CSF was used as opposed to our study in which single dose of G-CSF at the day of embryo transfer was injected.

Moreover several randomized studies using G-CSF supplementation in cases of repeated miscarriages, suggest a higher birth rate and fewer cases of pregnancy loss (19, 20). Also there are some studies that reported the G-CSF role on endometrial thickness improvement in patients with IVF failure (21). Lower fertility rate and higher spontaneous abortion rate was shown in G-CSF deficient mice (22-24). However In the present study the rate of abortion was not significantly different after usage of single dose of G-CSF compared with control group.

In the present study, the mean age of the participants was literally high with relatively high FSH and relatively low anti-mullerian hormone levels compared with other studies. However, any increase in the rate of pregnancy is very important in the treatment process. The possibility of G-CSF positive effects on pregnancy outcome presumed in a less adversely selected and especially younger patient population in larger studies.

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Conclusion

Our result demonstrates that pregnancy outcome was better in women with repeated IVF failure who are treated with G-CSF compared to the control group, but this difference was not stll statistically significant. Further studies with larger sample sizes and in younger women is recommended.

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Conflict of interest

No conflict of interest.

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