

# Immune Therapy to Improve Live Birth Rates in Patients Undergoing Intracytoplasmic Sperm Injection

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## ABSTRACT

**Background & Objective:** Granulosa cells, the endometrium, and the placenta all play a role in the secretion of G-CSF in the reproductive tract. G-CSF affects immunological regulation, which is crucial for enhancing pregnancy viability and maintenance. To evaluate the effects of granulocyte-colony stimulating factor subcutaneous injection on the miscarriage rate, ongoing pregnancy rate, and livebirth rate after single and multiple doses of G-CSF factor.

**Materials & Methods:** At the High Institute for Infertility Diagnosis and Assisted Reproductive Technologies at Al-Nahrain University, a prospective comparison study with a random sample selection was conducted from December 2021 to December 2022. It included 121 infertile women who had previously unsuccessful intracytoplasmic sperm injection (ICSI) procedures and who had finished ICSI protocols and reached the embryo transfer day. Patients were divided into three groups on the day of the embryo transfer: The non-G-CSF group (49 patients) received no additional treatment; the single-G-CSF group (31 patients) received a single subcutaneous injection of granulocyte-colony stimulating factor one hour after embryo transfer; and the multiple-G-CSF group (41 patients) received weekly injections of G-CSF until a fetal heartbeat could be detected, starting one hour after the embryo transfer.

**Results:** The multiple G-CSF group had better results in the miscarriage rate, ongoing pregnancy rate, and livebirth rate (23.5%, 34.1%, and 31.7%, respectively) when compared to the single G-CSF group (42.9%, 16.1, and 12.9%, respectively) and the non-G-CSF group (37.5%, 12.2%, and 10.2%, respectively).

**Conclusion:** Multiple subcutaneous G-CSF doses can improve the miscarriage rate, ongoing pregnancy rate, and live birth rate. A single dose of G-CSF for infertile women has no appreciable benefits.

**Keywords:** Infertility, Miscarriage rate, Live Birth Rate, G-CSF



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## Introduction

Assisted reproductive technology (ART), especially in vitro fertilization (IVF), is used to treat infertility. IVF, which is an expensive and time-consuming technology, still has a success rate of less than 40%. Many infertile couples who are experiencing physical, financial, and emotional stress are still unable to conceive despite undergoing IVF treatments (1).

According to the International Dictionary on Infertility and Fertility Care, the process of a hatched blastocyst attaching and then penetrating into the endometrium is known as implantation. The implantation process starts five to seven days after oocyte fertilization and typically results in the creation of a gestational sac (2). When an embryo reaches the receptive phase, the endometrium is viewed as a passive tissue that must facilitate implantation.

However, the receptive endometrium has undergone significant morphological and molecular alterations in response to the embryo (3). Gene expression profiles significantly alter in conjunction with the physical changes in the endometrium. In response to signals from the embryo, uterine cells adjust the expression of several genes, including transcription factors, alter the distribution of glycans, and sort integrin in different ways (4).

A well-known hematopoietic cytokine called granulocyte colony-stimulating factor (G-CSF) promotes the proliferation, differentiation, and activation of granulocyte line cells by interacting with their cell surface receptors (5). G-CSF has a wide spectrum of immunomodulatory effects, including the capacity to induce migratory activity and the survival

and regenerative abilities of numerous cellular elements in a dose-dependent way (6). G-CSF is secreted in the reproductive tract in three different ways: at the time of ovulation by the granulosa cells, which stimulate follicular growth, steroidogenesis, and activation of leucocytes necessary for ovulation; at the luteal phase by the endometrium, which causes vascular remodeling and decidualization; and finally, at gestation by the placenta, which supports pregnancy sustenance (7). Over the past 20 years, additional evidence of G-CSF's immune-regulatory activities, notably its impacts on T cell activity, has been obtained (8). G-CSF infusion in laboratory mice stopped the growth of a graft-versus-host reaction. The balance between the Th1 and Th2 subpopulations shifts toward Th2 as a result of G-CSF's inhibition of T-helper cell growth and differentiation. Interestingly, G-CSF promotes CD4+ and CD25+ regulatory T cells' production of interleukin-10, which raises graft macro-organism tolerance, with immunological tolerance being connected to T cell IL-10 production (9). In order to increase the rate of livebirth and decrease miscarriage rates in patients using assisted reproductive technologies, various researchers have suggested using G-CSF as a therapy option (10). The best method and timing for administration remain debatable (11); as a result, this study compares the effects of single and multiple doses of G-CSF.

## Methods

The present prospectively comparative study was approved by the High Institute for Infertility Diagnosis and Assisted Reproductive Technologies. All participants received detailed instructions about the administration of G-CSF and provided signed consent. This study included 121 infertile women ranging in age from 18 to 41, for a one-year period from December 2021 to December 2022.

### Inclusion criteria

Couples undergo embryo transfers with embryo grades 1 and 2, female factors, including anovulatory cycles and tubal blockage, Infertility caused by men, and unexplained infertility.

### Exclusion criteria

Acquired uterine anomalies (polyp, submucosal fibroid, intrauterine adhesion, and hydrosalpinx), contraindications for G-CSF, uncontrolled medical or endocrine disorder, and unwilling patients.

A complete clinical, surgical, gynecological, and obstetrical history is obtained. On day two of the

menstrual cycle, a full hormonal analysis (FSH, LH, E2, prolactin, and TSH) was performed. The patients received gonadotropin injections, with doses adjusted based on patient age, BMI, and antral follicle count. When the follicles reached 14 mm, they received GnRH antagonist injections (cetrotide 0.25 mg/day). When the follicles reached 18 mm, an ovulation trigger was given by HCG, and 35 hours later, oocyte retrieval was done. Then, two to three days later, the embryo transfer was done. On the day of embryo transfer, the patients were divided into three groups: the control group (49 patients) without any additional intervention; the single G-CSF group (31 patients) who received a single subcutaneous injection of granulocyte colony stimulating factor (Reliance®, Filgrastim TM, Life Sciences Ltd., India), which contains 300 µg/0.5 ml of recombinant human granulocyte colony stimulating factor solution in a prefilled syringe one hour after embryo transfer; and the multiple G-CSF group (41 patients) who receive multiple doses of G-CSF (the first injection was given one hour after embryo transfer; and subsequent injections were given weekly until the fetal heartbeat is detected; Luteal phase support and follow-up were done.

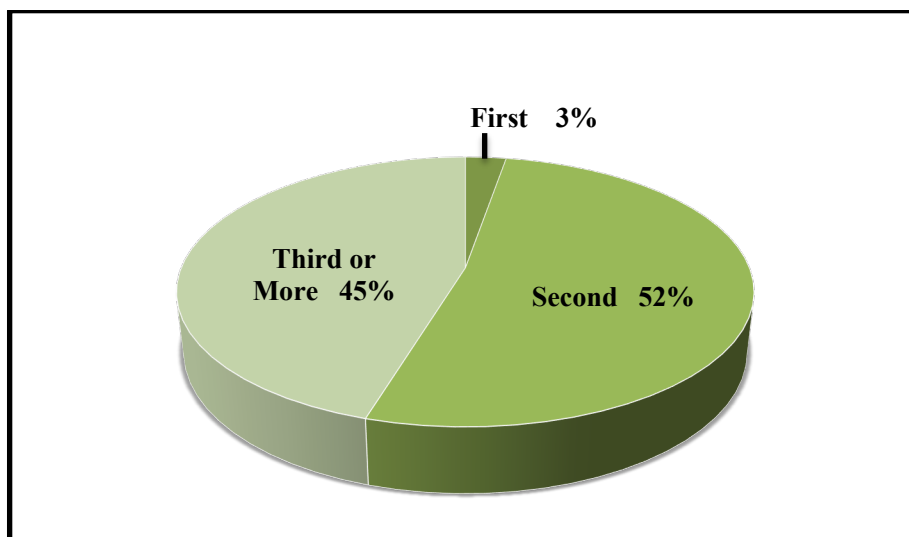
The ongoing pregnancy rate is defined as a positive fetal heart rate on an ultrasound performed at 12 weeks of gestation or later (12). The miscarriage rate was determined by dividing the number of pregnancy losses occurring up to the 24th week of pregnancy by the total number of patients with positive clinical pregnancy results (13). Any birth after 24 complete weeks of gestation per stimulation cycle is considered a live birth (14).

### Statistical analysis

The Statistical Package for Social Sciences version 26 (SPSS software, version 26, IBM, USA) and Microsoft Office Excel 2010 are used. Qualitative data was presented as numbers and percentages, while quantitative data was presented as the mean ± standard deviation. The groups were compared by applying the analysis of variance test (ANOVA) between three groups. Differences between the data were considered significant if the  $P \leq 0.05$

## Results

The mean age of females who participated in the study was  $30.67 \pm 4.48$ ; 52% of patients have had one previous failed ICSI trial, and 45% of patients have had two or more previous failed ICSI trials, as shown in [Figure 1](#).



**Figure 1.** ICSI Trial

The miscarriage rate, ongoing pregnancy rate, and livebirth rate for the three groups are shown in [Table 1](#), which shows that there were significant differences

between the three groups in the three parameters that were used as the final outcome in this research, as seen from *P* values (0.050, 0.029, and 0.0021, respectively).

**Table 1.** ICSI Outcomes

ICSI Outcome%(N)	Non G-CSF N= 49	Single G-CSF N= 31	Multiple G-CSF N= 41	<i>p</i> value C
Miscarriage Rate	37.5 % (3/8)	42.9 % (3/7)	23.5 % (4/17)	0.050
Ongoing Pregnancy Rate	12.2 % (6/49)	16.1 % (5/31)	34.1 % (14/41)	0.029
Live Birth Rate	10.2 % (5/49)	12.9 % (4/31)	31.7 % (13/41)	0.0021

## Discussion

Non-hematopoietic cell types include endothelium, placenta, trophoblastic, and granulosa lutein cells that can have G-CSF receptors (15). G-CSF appears to influence the expression of genes crucial for the implantation process in the endometrium, such as those involved in cellular adhesion pathways, local immune control, and endometrial vascular remodeling. By enhancing implantation and endometrial thickness, G-CSF promotes successful conception (19 in this study, G-CSF was used in patients with at least one prior failure (97% of patients had at least one failure, as shown in [Figure 1](#)) due to its involvement in improving live birth rate and decreasing miscarriage rate. As shown in [Table 1](#), the current investigation discovered that multiple G-CSF group had considerably improved pregnancy rates (both chemically and clinically). These results are in line with those of other researchers who claim that G-CSF has been shown to be crucial to both the implantation of embryos and the maintenance of pregnancies (16). Furthermore, in a randomized controlled study on patients with recurrent implantation failure, Scarpellini and Sbracia discovered that when a daily dose of 60 mg of GCSF was initiated and maintained for an additional 40 days

after a positive pregnancy test, the miscarriage rate decreased (17). A single G-CSF dose is ineffective in supporting ICSI outcome metrics, as shown in [Table 1](#). This is consistent with the findings of Zeyneloglu and colleagues, who claim that G-CSF is a promising and secure treatment to raise the pregnancy rate in patients with implantation failure, with double G-CSF doses appearing to be more effective than a single dose (18). In contrast to a single dose, Sen and Khastgir's research indicates that a doubled dose of G-CSF greatly increases the pregnancy rate (19). Scarpellini and Sbracia conducted two studies in 2011 and 2013 and found that the use of G-CSF subcutaneously daily from the day of embryo transfer for forty days improved the implantation and ongoing pregnancy rates in the first study and significantly increased T regulatory cells in the decidua of patients treated with G-CSF subcutaneously daily for ten days, which explains the beneficial effect of multiple G-CSF injections (20, 21). This is in disagreement with Aleyasin et al. (2016), who conducted a multicenter, randomized, controlled experiment and found that a single dose of 300 µg of G-CSF provided subcutaneously 1 h before the embryo transfer can increase implantation and pregnancy rates

in infertile women with repeated IVF failures (22). While Torky and colleagues' study found that intrauterine administration of G-CSF at the time of ova pickup can improve pregnancy and implantation rates but has no effect on miscarriage rates, but this improvement is not significant (23). This difference may be due to differences in the timing or route of administration, or it could be due to the fact that the single G-CSF was insufficient to support and maintain the pregnancy, as in this study. Further study on the uses of G-CSF in infertile women was needed to find the optimal route, dose, and timing of administration.

## Conclusion

Studies have revealed that giving a single dose of G-CSF subcutaneously to infertile women has no appreciable benefits. Multiple G-CSF dosages can decrease miscarriage rate and enhance ongoing pregnancy and livebirth rates.

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## Author Contribution

The research was carried out by Aseel ALFIL, under the supervision of Lubna Al-Anbari.

## Ethical Clearance

The Ethical Approval Committee approved the study.

## Conflict of Interest

There is no conflict of interest.

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