ORIGINAL ARTICLES

Efficacy of Subcutaneous Administration of Granulocyte Colony-Stimulating Factor in Patients with Recurrent Implantation Failure in Frozen-Thawed Embryo Transfer Cycles

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Background: *To investigate the effect of the subcutaneous administration of G-CSF on pregnancy outcomes in patients with RIF before and after FET.*
Methods: *This retrospective study included 170 patients who underwent FET at the Center for Reproduction and Genetics of The First Affiliated Hospital of USTC, Hefei, China, from January 1, 2021, to December 31, 2022. Seventy-five of them were administered G-CSF, and the others received no specific treatment. The clinical pregnancy rate, embryo implantation rate, biochemical pregnancy rate, abortion rate, and live birth rate of both groups were calculated by SPSS software.*
Results: *The clinical pregnancy rate, embryo implantation rate, biochemical pregnancy rate, abortion rate, and live birth rate had no difference between the G-CSF group and the control group ($P \geq 0.05$).*
Conclusion: *The subcutaneous administration of G-CSF had no positive effect on pregnancy outcomes in RIF patients who underwent FET cycles. However, more research is needed.*

BACKGROUND

RIF, a major challenge in the field of assisted reproduction, occurs in approximately 10% of patients who undergo in vitro fertilization (IVF) or Intracytoplasmic sperm injection (ICSI).1 Although the current definition of RIF varies, RIF is generally defined as failure to achieve clinical pregnancy after the transfer of at least four high-quality embryos in at least three fresh or frozen cycles by IVF or ICSI in women younger than 40 years of age.2 The causes of RIF are complex and include both maternal and embryonic factors. Maternal factors include abnormalities in the anatomical structure of the reproductive organs, a prethrombotic state, endometrial lesions, autoimmune diseases, an abnormal immune microenvironment at the maternal–fetal interface, and other unknown causes.[3]

G-CSF is a glycoprotein derived from various tissues and cells, such as fibroblasts, monocytes, macrophages, natural killer (NK) cells, placental cytotrophoblasts, endometrial glandular cells and follicular cells.[4,5] It plays an important role in promoting the proliferation and differentiation of precursor cells of the neutrophilic granulocyte cell lineage and is widely used in hematological diseases firstly. Since then, its biological roles, including roles in immune responses, cell proliferation, survival, and cancer pathogenesis, have been studied extensively.[6,7] Recent studies have shown that G-CSF also plays an important

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important role in human reproductive achievement. Several studies have shown that G-CSF can affect oocyte maturation.[8]

One cannot ignore the fact that G-CSF also plays a role in the process of embryo implantation. G-CSF has been reported to regulate the expression of vascular endothelial growth factor (VEGF), leukemia inhibitory factor (LIF), and proliferative cell nuclear antigen (PCNA) to promote endometrial proliferation and angiogenesis.[9] Besides, G-CSF is an immunomodulatory factor that to modulate the balance between the Th1 and Th2 immune responses, which is important for regulating immunotolerance within the microenvironment at the maternal–fetal interface.[10,11] In addition, G-CSF can regulate the invasion and migration of trophoblasts by activating the PI3K/Akt and Erk1/2 signaling pathways.[12] However, the results of recent clinical studies on the effectiveness of G-CSF against RIF are controversial. Zeyneloglu et al. reported that G-CSF treatment could improve the pregnancy rate of women with RIF compared with that of women in the control group.[13] However, some later studies showed inconsistent results, so more research is needed.[14,15]

The mode and time of administration may be factors affecting the effectiveness of G-CSF in regulating embryo implantation. In our study, we aimed to investigate the effect of subcutaneous administration of G-CSF 75 U daily or 150 U every other day 5 days before FET and after FET on the clinical pregnancy outcomes of RIF patients. We hope to provide some information for the application of G-CSF in patients with RIF.

MATERIALS AND METHODS

Study design and population

In this retrospective cohort study, we analysed the data of RIF patients who underwent frozen-thawed embryo transfer again at the Center for Reproduction and Genetics of The First Affiliated Hospital of University of Science and Technology of China (USTC), Hefei, China, from January 1, 2021, to December 31, 2022. The primary outcome was clinical pregnancy rate; comparisons of patients who received and did not receive subcutaneous G-CSF were performed. The study was approved by the Institutional Review Board of The First Affiliated Hospital of USTC, and all women provided informed consent.

The inclusion criteria were RIF patients under the age of 40 years who failed to achieve clinical pregnancy after at least four high-quality embryos in at least three fresh or frozen cycles by IVF or ICSI were transferred and who underwent FET again. The exclusion criteria were as follows: (1) the karyotype of the patient or her spouse was abnormal; (2) the patient was taking other drugs that may promote embryo implantation, such as human chorionic gonadotropin (hCG); (3) the patient was given intrauterine injections of G-CSF; (4) the patient had used donor sperm or eggs; (5) the patient had uterine defects; or (6) the patient had a chronic disease. A total of 170 patients were enrolled in this study, including 95 in the control group and 75 in the G-CSF group.

Treatment protocol

According to the patient's own condition, the appropriate endometrial preparation protocols were selected and mainly included the natural cycle (NC), mild ovarian stimulation (mild-OS), hormone replacement treatment (HRT) with gonadotropin-releasing hormone (GnRH) analogue suppression, and HRT without GnRH-a. For the NC, transvaginal ultrasonographic monitoring was usually started on days 8-10, and endocrine monitoring was performed until the day of natural or hCG-triggered ovulation. For mild-OS, 2.5–5 mg of letrozole was given beginning on the 5th day of the menstruation cycle for 5 days. Depending on follicular growth, low-dose menotropin for injection (HMG) was subsequently to the regimen. For HRT without GnRH-a, estradiol was administered from the 2nd to 3rd day of the menstrual cycle until endometrial thickness reached a certain level. For HRT with GnRH-a, 3.75 mg of GnRH-a (leuprorelin acetate microspheres for injection) was given at the 2nd to 3rd day of the menstrual cycle. Estradiol was added as HRT without GnRH-a from 28-35 days after GnRH-a administration. Then, progesterone support treatment was given in all 4 protocols. The endometrial thickness was measured on the first

day of progesterone support treatment.

In the G-CSF group, subcutaneous administration of G-CSF 75 U daily or 150 U every other day 5 days before FET and after FET, and the control group received no specific treatment.

In our subsequent analysis, we divided the embryos into high-grade embryos and other transferable embryos. According to the Istanbul Consensus, high-grade embryos were defined as grade I and II embryos, and other transferable embryos included grade III embryos.[16] In general, cleavage-stage embryos were transferred 3 days after progesterone support treatment, and blastocysts were transferred 5 days after progesterone support treatment.

Outcome

β-hCG was measured 14 days after cleavage-stage embryo transfer or 12 days after blastocyst transfer. Embryo implantation and clinical pregnancy were detected by transvaginal ultrasonography one month after embryo transfer. Biochemical pregnancy diagnosis was defined as a β-hCG ≥ 25 IU/L and no gestational sac on transvaginal ultrasonography, followed by pregnancy termination. Successful implantation was defined as detection of a gestational sac by transvaginal ultrasonography. Abortion was defined as a spontaneous abortion or embryo damage after a diagnosis of clinical pregnancy.

Statistical analysis

All the data analyses were performed using SPSS version 26.0 (IBM, Armonk, NY, USA). Quantitative data are expressed as the mean ± standard deviation (SD) or M (P25, P75). Qualitative data are expressed as frequencies and proportions. Differences in infertility type, infertility cause, embryo transfer protocol, embryo type, embryo grade, and embryo number among groups were analysed with the chi-squared test ($\chi 2$). Differences in infertility duration among groups were analysed by the Wilcoxon Mann–Whitney test. Differences in age, body mass index (BMI), and endometrial thickness among groups were compared by independent Student's t test. Logistic regression analysis was performed to assess the odds ratios of factors related to clinical pregnancy rates between the two groups. The primary outcome was the clinical pregnancy rate. The secondary outcomes were biochemical pregnancy rate, embryo implantation rate, abortion rate, and live birth rate. These variables were analysed by means of the $\chi 2$ test. Statistical significance was defined as a P value of less than 0.05.

RESULTS

Baseline characteristics of the patients

The baseline characteristics of the control and G-CSF groups are presented in Table 1. There were no significant differences in female age, male age, female BMI, infertility type, infertility duration, infertility cause, embryo transfer protocol, embryo type, embryo quality, or number of transferred embryos. However, endometrial thickness differed between the control and G-CSF groups ($P < 0.05$), with the thickness being higher in the control group than in the G-CSF group (9.73 mm and 9.21mm, respectively).

Clinical outcomes

Comparisons of the clinical outcomes of the two groups are presented in Table 2. There was no difference in the clinical pregnancy rate ($P > 0.05$). There were 35 of 95 patients (36.8%) in the control group and 29 of 75 patients (38.7%) in the G-CSF group who achieved clinical pregnancy. Additionally, the comparisons of several secondary outcomes, namely, biochemical pregnancy rate, embryo implantation rate, abortion rate, and live birth rate, showed that there were no differences between the two groups.

There were confounding biases, such as differences in endometrial thickness, between the two groups. To exclude the effect of confounding bias on the clinical outcomes, we selected factors with $P < 0.2$ for further multiple logistic regression analysis. As shown in Table 3, the clinical pregnancy rate was not significantly affected by endometrial thickness, BMI, or infertility duration in this study ($P > 0.05$).

Table 1: Baseline characteristics of the patients in this study.

Characteristics	Control(n=95)	G-CSF(n=75)	Test	*P*-value
Female age (Y) Mean±SD	33.21±3.51	33.23±4.05	*t* =-0.027	0.978
Male age (Y) Mean±SD	34.46±4.61	34.17±5.18	*t* =0.385	0.701
BMI (kg/m2) Mean±SD	22.48±2.97	21.84±2.49	*t* =1.511	0.133
Infertility type (n, %)			χ^2=0.132	0.716
Primary	47 (49.5%)	35 (46.7%)		
Secondary	48 (50.5%)	40 (53.3%)		
Infertility duration (Y)			*z=1.380*	0.168
M (P_{25}, P_{75})	4 (3, 5)	4 (2, 5)		
Infertility cause (n, %)			χ^2=1.073	0.928
Tubal	69 (72.6%)	52 (69.3%)		
Ovarian	2 (2.1%)	2 (2.7%)		
Endometriosis	6 (6.3%)	6 (8%)		
Male	14 (14.7%)	10 (13.3%)		
Other	4 (4.2%)	5 (6.7)		
Embryo transfer protocol (n, %)			χ^2=2.685	0.443
NC	8 (8.4%)	2 (2.7%)		
mild-OS	16 (16.8%)	14 (18.7%)		
HRT with GnRH-a	41 (43.2%)	32 (42.7%)		
HRT without GnRH-a	30 (31.6%)	27 (36%)		
Endometrial thickness (mm)			t =2.142	0.034
Mean±SD	9.73±1.57	9.21±1.58		
Embryo type (n, %)			χ^2=0.074	0.785
D3	61 (40.9%)	49 (42.6%)		
D5	88 (59.1%)	66 (57.4%)		
Embryo grade (n, %)			χ^2=1.370	0.242
High-grade	103 (69.1%)	87 (75.7%)		
Other	46 (30.9%)	28 (24.3%)		
Embryo number (n, %)			χ^2=0.209	0.648
1	41 (42.3%)	35 (46.7%)		
2	54 (56.8%)	40 (53.3%)		

Table 2: Clinical outcomes

Outcomes	Control (n=95)	G-CSF (n=75)	χ2	*P*-valve	OR (95%CI)
Biochemical pregnancy rate	4 (4.2%)	5 (6.7%)	0.133	0.715	0.62 (0.16, 2.38)
Embryo implantation rate	40 (26.8%)	30 (26.1%)	0.019	0.89	1.04 (0.60, 1.81)
Clinical pregnancy rate	35 (36.8%)	29 (38.7%)	0.059	0.807	0.93 (0.50, 1.73)
Abortion rate	4 (11.4%)	3 (10.3%)	< 0.001	> 0.99	1.12 (0.23, 5.46)

Table 3: Multiple logistic regression analysis

Characteristics	b	Wald	*P*-value	OR (95%CI)
BMI	0.06	1.002	0.317	1.06 (0.94, 1.20)
Infertility duration (Y)	-0.114	1.980	0.159	0.89 (0.76, 1.05)
Endometrial thickness (mm)	0.119	1.368	0.242	1.13 (0.92, 1.37)

DISCUSSION

In our current study, considering that G-CSF can improve the immune microenvironment at the maternal–fetal interface, promote endometrial angiogenesis, and increase trophoblast invasion, we aimed to investigate the administration time of G-CSF 5 days before FET and after FET. The results showed that in RIF patients, subcutaneous injection of G-CSF had no significant positive effect on the embryo implantation rate or clinical pregnancy rate, which was consistent with previous studies.[15] We also found that G-CSF administration at this time did not affect the abortion rate or live birth rate, indicating that G-CSF generally had no obvious side effects on embryonic development. Several studies have investigated the safety of G-CSF and reported that there were no differences in the teratogenic effect on the developing fetus between the G-CSF group and the control group.[17,18] In addition, the administration of G-CSF did not increase obstetric or neonatal complications.[19,20] However, there were some nonspecific side effects, such as nausea and vomiting, anorexia, and headache, in patients treated with G-CSF.[8]

G-CSF is involved in regulating multiple processes related to reproduction, including improving ovarian function, promoting endometrial growth, and increasing embryo development and post-transfer survival. For example, G-CSF could significantly prevent luteinized unruptured follicle syndrome[21] Jinno et al. reported that G-CSF could significantly increase AMH levels in patients with poor ovarian reserve.[22] Kunicki et al. showed that G-CSF could increase endometrial thickness.[23] In recent years, there has been more research, including retrospective and prospective studies, that has focused on the application of G-CSF in patients with RIF. Such research can be further divided into G-CSF administration in fresh embryo transfer cycles and that in frozen embryo transfer cycles. Zeyneloglu et al. showed that G-CSF treatment increased the clinical pregnancy rate in RIF patients undergoing fresh embryo cycles.[13] However, negative results were reported in another RCT.[24] One study of frozen embryo transfer cycles revealed that G-CSF had no effect on increasing the clinical pregnancy rate of RIF patients.[15]

However, another meta-analysis showed that G-CSF could increase the clinical pregnancy rate in frozen embryo transfer cycles in RIF patients.[5] To date, the efficacy of G-CSF in RIF patients remains controversial.

Notably, the administration of G-CSF to infertile patients includes subcutaneous injection and intrauterine injection, and different administration methods may also be one of the reasons for the inconsistent conclusions drawn.[5, 24, 25] In addition, there is no unified standard for the time and dose of G-CSF administration, which are possible factors causing differences in the efficacy of G-CSF.

In our study, we clearly restricted the administration method, administration time, and dose of administration, which differed from previous studies, and this may have contributed to the difference between our results and those of some previous studies. In addition, the small number of patients included in our study may have affected the outcome.

Thus, more high-quality, larger and multicentre RCTs are needed to provide more authoritative evidence for clinical practice.

DECLARATIONS

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The datasets used during the current study are available from the corresponding author on reasonable request.

Conflicts of Interest

The authors have declared no conflicts of interest.

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Authors' contributions

MJQ was involved in data collection, analysis, interpretation, manuscript writing, and funding acquisition. CC was involved in data collection, analysis, and funding acquisition. YM was involved in study conception and design, manuscript writing and editing. All authors approved the final version of the manuscript.

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