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A Comparison of Endometrial Thickness and Pregnancy Outcomes in Two Methods of Intrauterine Injection and Subcutaneous Injection of GCSF in Infertile Women Candidates for IVF

Zahra Rezaei, Khadijeh Adabi, Adele Sadjadi*

Department of Obstetrics and Gynecology, Yas Women General Hospital, Tehran University of Medical Sciences, Tehran, Iran

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Corresponding Information:
Adele Sadjadi,
Department of Obstetrics and Gynecology, Yas Women General Hospital, Tehran University of Medical Sciences, Tehran, Iran
Email: adeleh.sadjadi@gmail.com
Tel: +98 2142160000

ABSTRACT

Background & Objective: An endometrial thickness of less than 7 mm adversely affects pregnancy outcomes. There is also ample evidence of the impact of granulocyte colony-stimulating factor (G-CSF) on treatment-resistant thin endometrium. Therefore, this study compares the effects of intrauterine and subcutaneous injections of G-CSF on increasing endometrial thickness (ET) and pregnancy outcomes in infertile women who were candidates for in vitro fertilization (IVF).

Materials & Methods: In the current randomized, double-blind clinical trial, 34 patients with a history of failed IVF cycles and treatment-resistant thin endometrium were randomized into two routes of G-CSF injection, i.e., intrauterine and subcutaneous, by using a random number table method. Outcomes including ET and chemical and clinical pregnancy rates were compared in two groups.

Results: ET increased significantly in both groups after G-CSF administration (intrauterine and subcutaneous); however, this increase in thickness was significantly greater in the intrauterine injection group than in the subcutaneous injection group. Pregnancy outcomes were similar in two groups. Drug side effects were significantly lower in the intrauterine injection group than in the subcutaneous injection group.

Conclusion: G-CSF can significantly increase ET in cases of repeated IVF failure, and intrauterine injection of this drug was more effective than subcutaneous injection.

Keywords: Treatment-resistant thin endometrium, Granulocyte colony-stimulating factor, In vitro fertilization

Introduction

All through the world, about 15% of couples struggled with infertility **(1)**. Assistance Reproductive Technology (ART) can be so helpful to solve this problem but Several factors can affect in vitro fertilization (IVF) success rates. The most important independent variables are woman's age, anti-Müllerian hormone (AMH) level, and number of transferred embryos and their quality **(2-4)**. Studies have also shown that an endometrial thickness (ET) of less than 7 mm negatively influences pregnancy outcomes **(5, 6)**.

Further, some evidence suggests that immunological mechanisms in the endometrium are critical to the implantation process **(7)**. Some researchers have noted that growth factors, hormones, and cytokines synthesized by decidual cells are involved in the implantation process **(8)**.

Previous studies have demonstrated that granulocyte colony-stimulating factor (G-CSF) stimulates the proliferation and differentiation of neutrophilic granulocytes, affects macrophages in decidual cells, and ultimately influences the implantation **(9)**.

Recent studies have indicated that intrauterine injection of G-CSF is effective in treatment-resistant infertile women **(10, 11)**. G-CSF is a cytokine that stimulates the proliferation and differentiation of neutrophilic granulocytes **(12)**.

This drug improves fetal implantation, affects decidual macrophages, ovulation, ovarian function, and granulosa cell function, boosts the ovarian response to gonadotropin in patients with a poor response, and reduces recurrent miscarriages. It is also involved in the recovery of primary endometrial lesions and suppresses autoimmune reactions **(13-21)**.

In addition to what was noted, G-CSF as a biomarker for the implantation potential of an egg or embryo can predict IVF outcomes **(22, 23)**.

Given the above-mentioned description and considering the limited studies evaluated the effects of intrauterine injection of G-CSF, in this study, we attempt to evaluate and compare the effects of intrauterine and subcutaneous injections of G-CSF on increasing ET and pregnancy outcomes in infertile women who were candidates for IVF.

Materials and Methods

This double-blind trial study was conducted on 34 women who were candidates for frozen IVF cycles in Yas Hospital. This study was approved by the Ethical Committee of Tehran University of Medical Sciences (ethical code: IR.TUMS.MEDICINE.REC.1396.2811), and all patients signed informed consent.

Patients with a history of failed cycles and ET of less than 7 mm (measured by ultrasound) were included based on the inclusion and exclusion criteria. In the current study, women aged 18 to 40 years, who had at least two failed IVF cycles with a minimum of three suitable embryos for transfer, were enrolled.

Patients were excluded from the study in the case of having contraindications for G-CSF injection, suffering from any systemic diseases, dealing with Asherman's syndrome, fibroids, and polyps detected by hysteroscopy, and being unwilling to participate in this study. After entering the study, the patients were divided into two groups using a random number table method.

At the beginning of the study, all the patients underwent laboratory tests, including fasting glucose sugar, lipid profile, and liver and thyroid function tests, to rule out systemic diseases.

Estradiol valerate tablets (2 mg; Aburaihan Pharmaceutical Company, Tehran, Iran) were administered daily for all the patients from the second day of the menstruation cycle, and then the estradiol dose was increased to 10 mg per day. On the 12th day of the menstruation cycle, transvaginal ultrasound was performed, and ET was measured in the thickest part and the longitudinal view. In the case of thickness less than 7 mm, ultrasound was again conducted on the same day by another radiologist, and the mean values measured as ET were recorded in the patient's medical file, and the patient was treated with G-CSF.

In the first group, 300 µg of G-CSF (Neupogen, F.Hoffmann-LA Roche, Swiss) with a volume of 1 mLcc was transvaginally injected into the uterus by intrauterine insemination (IUI) catheters. For making it a double-blind study, a placebo (normal saline with a similar volume) was administered simultaneously and subcutaneously. In the second group, the same dose of G-CSF (300 µg per 1 mLcc) was injected subcutaneously, and the intrauterine placebo was injected. All the drugs were prepared and coded for injection blindly.

ET was assessed by ultrasound 72 hours after G-CSF injection. All the patients' sonographies were performed by a radiologist between 9 am AM and noon, who did not know the grouping and method of drug injection. If ET was more than 7 mm, after receiving progesterone for three days, a three-day embryo was transferred. In the case of no increase in ET (above 7 mm), the cycle was canceled.

The rate of chemical pregnancy in the two groups was evaluated two weeks after the embryo transfer by measuring the serum β-subunit human chorionic gonadotropin BHCG levels (using Roche kit, electrochemiluminescence immunoassay (ECLIA) method, and Cobas 411 device, considered positive when the value was more than 25). The rate of successful embryo implantation was tested four weeks after the embryo transfer by transvaginal sonography, and the clinical pregnancy evaluations were performed six weeks after the embryo transfer by abdominal sonography.

Results

In this study, 34 women with a history of two failed IVFs were studied in two groups. Underlying variables, such as patients' mean age, body mass index (BMI), AMH, ET, and infertility type, were studied separately in the two groups; however, no significant difference was observed in these variables between the two groups (Table 1).

Table 1. The comparison of the basic variables in the two groups

Variables	Intrauterine GCSF	Subcutaneous GCSF	P-value
Age	30.94±4.30	31.12±5.15	0.914
BMI	25.46±2.14	24.86±1.75	0.425
AMH	2.01±0.51	2.21±0.51	0.250
ET	5.67±0.51	5.86±0.61	0.340
Infertility Type			
Primary	14 (82.35)	12 (70.59)	0.267
Secondary	3 (17.65)	5 (29.41)	

Abbreviations: BMI: body mass index, AMH: anti-müllerian hormone, ET: endometrial thickness.

The mean ET of both groups in the pre- and post-injection sections was compared using a paired *t* test; the analysis showed a significant increase in both groups (P<0.001). Accordingly, ET increased by 42.2% in the intrauterine injection group and 23.6% in the subcutaneous injection group (P=0.002; see Table 2).

Table 2. The changes in the mean endometrial thickness after GCSF injection in the two groups, i.e., intrauterine injection and subcutaneous injection

Groups	Pre ET	Post ET	P-value
Intrauterine GCSF	5.67±0.51	8.07±0.64	<0.001
Subcutaneous GCSF	5.86±0.61	7.24±0.76	<0.001

Abbreviations: GCSF: Granulocyte colony-stimulating factor, ET: endometrial thickness

Two weeks after the embryo transfer, the rate of chemical pregnancy was assessed by BHCG. Five cases (29.4%) in the intrauterine injection group and four cases (23.5%) in the subcutaneous injection group got pregnant (P=0.348).

Six weeks after the embryo transfer, the rate of clinical pregnancy in the patients was evaluated. In this regard, three patients (17.64%) got pregnant in the intrauterine injection group, and two patients (11.76%) got pregnant in the subcutaneous injection group (P=0.628).

The patients were also evaluated for drug side effects. Two patients (11.8%) in the intrauterine injection group and nine patients (52.9%) in the subcutaneous injection group had a drug side effect (P=0.010).

Considering the results of the present study and comparing the pregnant and non-pregnant patients showed that the AMH and ET in pregnant patients were significantly higher than in non-pregnant women (Table 3).

Table 3. The comparison of AMH and ET between the pregnant and non-pregnant patients

Variables	Pregnant women	Non-pregnant women	P-value
AMH	2.6	1.97	0.001
ET	8.44	7.66	<0.001

Abbreviations: AMH: anti-müllerian hormone, ET: endometrial thickness.

Discussion

The results of the current study showed that intrauterine injection of G-CSF could significantly increase ET compared to subcutaneous injection. However, although the rate of chemical and clinical pregnancies was higher in the intrauterine injection group than in the subcutaneous injection group, this difference was not significant.

The rate of clinical pregnancy after the embryo transfer boosted with increased ET **(4-9)**. Most studies have indicated that the minimum effective ET for implantation is 7 mm (10-14). Although most patients undergoing IVF reach this minimum ET with routine treatment protocols, a small number of patients do not reach normal ET and require more therapeutic interventions. Therefore, it is very desirable to use a method that can increase ET in such patients.

For the first time, Gleicher *et al.* used intrauterine G-CSF in four patients who did not reach the appropriate ET after preparation **(11)**. In the mentioned study, after intrauterine injection of G-CSF, ET reached at least 7 mm in all subjects, who were previously resistant to estrogen and vasodilators. In the present study, ET of the patients who received intrauterine G-CSF injections was more than 7 mm, which is consistent with the above-mentioned study.

In a study conducted by Barad *et al.*, intrauterine injection of G-CSF did not have positive effects on IVF outcomes, and ET and clinical pregnancy rates did not differ significantly between the two groups **(24)**. However, in our study, although ET was significantly higher in the intrauterine injection group than in the subcutaneous injection group, the pregnancy outcomes did not differ between the two groups. These findings are consistent with Barad *et al.'s* study. It is important to note that patients' mean age in the mentioned study was about 14 years more than that of the patients in the present study.

In a study carried out by Eftekhar *et al.*, in which G-CSF intrauterine injection was administered, ET was not significantly different between the two groups; however, the rate of clinical pregnancy was significantly higher in the group that had G-CSF intrauterine injection **(25)**.

One of the limitations of the current study was the small number of participants. It is recommended to

increase the sample size in future studies, which may cause a significant difference between the two groups in terms of the clinical pregnancy rate.

Conclusion

G-CSF can significantly increase ET in cases of repeated IVF failure. Intrauterine injection of this drug was more effective than subcutaneous injection.

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Conflict of Interest

Authors declared no conflict of interests.

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