Effect of granulocyte colony stimulating factor on pregnancy rate in women with unexplained infertility after intrauterine insemination: a randomized clinical trial

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Abstract

Background The cause of infertility has not been found in unexplained infertile patients,, and perhaps one of the possible reasons is impairment of fetal implantation, as well as the multiple role of GCSF in improving implantation and quality of blastocyst. Therefore, the aim of this study was to investigate the role of GCSF in the pregnancy rate of patients undergoing IUI.

Methods The patients with unexplained infertility were divided into two groups: one group was received GCSF in their IUI cycle and the other group had the routine IUI. Both groups were stimulated by letrozole, metformin, and monotropin during the cycle. When at least one follicle was greater than 18 mm, 5000 IU hCG intramuscularly was administered for ovulation induction and IUI was performed 34–36 hours later. In intervention group, 300 µg GCSF subcutaneously administrated in two days after IUI. Biochemical pregnancy rate was evaluated two weeks after IUI and clinical pregnancy rate was identified by the presence of a gestational sac on ultrasonography 8 weeks after IUI.

Results There was no significant difference in demographic and clinical characteristics between the two groups. The chemical pregnancy rate(16.3% vs 12.2%) and the clinical pregnancy rates (16.3% vs 8.3%) were improved in patients receiving GCSF compared to controls, but these differences was not significant (P = 0.56) and (P = 0.21).

Conclusion Systemic administration of a single dose of 300 µg GCSF subcutaneously two days after IUI may slightly improve clinical pregnancy rate in patients with unexplained infertility. Nevertheless, our findings do not support routine use of G-CSF in unexplained infertility women with normal endometrial thickness.

Background

Infertility refers to the inability of couples in pregnancy after a year of regular intercourse without contraception methods[1]. It is estimated that 15% of couples are seeking medical help for infertility and it seems that the origin of these problems is divided equally between men and women[2]. Unfortunately, due to the raw diagnostic tests available to identify potential apostle, the causes of infertility are not determined in half of the cases which named unexplained infertility. In these couples the routine semen analysis is within the reference values, and the definitive female infertility factor has not been identified[3]. Some patients have not been justified in endometrial function, which leads to the defect of the dialogue between the fetus and endometrium and may lead to implantation failure[4]. After 17 beta-estradiol priming for endometrial development and subsequent exposure to progesterone, the endometrium is receptive in limited period of time, which named the window of implantation. It appears to be between 19 and 21 days of 28-day cycle. Molecular and structural changes occur in endometrium during window of implantation. Implantation requires the harmonious development of blastocyst and endometrium able to respond to the signal of blastocyst[5]. A number of factors produced by endometrium during window of implantation considered as molecular markers of endometrial receptivity.
Many cytokines are involved in implantation. Interleukin 1, 11, 6, 10, 15, 18, and leukemia inhibitor factor (LIF), Tumor necrosis factor (TNF), transforming growth factor beta (TGF-B), colony stimulating factor (CSF) have an important role in implantation[6].

GCSF is a member of the hematopoietic growth factor family that produced by hematopoietic cells. Also, it is produced by multiple non-hematopoietic cells including osteoblasts, smooth muscles, endothelium and ovaries[7]. GCSF is produced at the maternal-fetal interface during embryo implantation and is the main part of the uterine-cytokine network that is needed to create and maintain pregnancy. This cytokine and other members of the CSF are involved in creating a dominant TH2 environment that is needed to create and maintain a local immune environment for a successful implantation[8].

Some studies have shown that systemic administration of GCSF in women with spontaneous abortions and repeated implantation failures can improve fertility outcomes[9]. Other studies have also reported that intrauterine infusion of GCSF was successful in women with thin endometrial (endometrial thickness less than 7 mm) and women with repeated implantation failures[10]. This could be due to the role of GCSF in improving endometrial thickness[11].

Given that, the cause of infertility has not been found in unexplained infertile patients,, and perhaps one of the possible reasons is impairment of fetal implantation, as well as the multiple role of GCSF in improving implantation and quality of blastocyst. Therefore, the aim of this study was to investigate the role of GCSF in the pregnancy rate of patients undergoing IUI.

**Methods**

**Trial Design & participants**

This study was a randomized clinical trial registered prospectively at the Iranian Registry of Clinical Trials (www.irct.ir: IRCT20160524028038N4). This study was conducted among 98 women with unexplained infertility aged 18–37 years who referred to the infertility clinic of Yasuj University of Medical Sciences, Yasuj, Iran, from July to December 2019. The main inclusion criteria were inability to get pregnant despite having frequent, unprotected intercourse for at least a year in absence of female and male factors. The study excluded the patients who have no desire for cooperation and the patients who have an inadequate endometrium (<7 mm) on the day of hCG injection.

**Ethics approval and consent to participate**

The study was followed the Declaration of Helsinki Guideline and approved by the ethics committee of Yasuj university of medical sciences, Yasuj, Iran. Written consent was obtained from all participates.

**Study protocol**

The patients with unexplained infertility were randomly divided into two groups: one group was received GCSF in their IUI cycle and the other group wasn’t received GCSF. For all patients, letrozole (Iran hormone,
Tehran, Iran) at a dose of 2.5 mg and clomiphene at a dose of 100 mg were prescribed per day from the third day of menstruation for 5 days and one vial of menotropine (Karma HMG, Homapharmed, Tehran, Iran) per day from 8 to 10 days of menstrual cycle. The patients were then evaluated during 12 to 16 days of menstrual cycles for the response of the drug, the size of the follicles reached in the ovary and the thickness of the endometrium by vaginal ultrasonography using a 6–9 MHz convex-array transducer (Ultrasonix RP, Vancouver, BC, Canada). With the observation of at least one mature follicle (≥18 mm), 5,000 units of HCG (Karma HCG, Homapharmed, Tehran, Iran) were injected. IUI was done 36 hours later. In intervention group 300 ug GCSF (Neupogen, Roche) subcutaneously administrated in two days after IUI. Biochemical pregnancy rate was evaluated two weeks after IUI. The clinical pregnancy was identified by the presence of a gestational sac on ultrasonography 5 weeks after HCG injection.

**Intrauterine insemination (IUI)**

Sperm preparation was done by density gradient which is highly effective at isolating motile sperm for insemination. Density gradient was done based on factory instruction. We make the lower (90%) phase and upper (45%) phase gradient by AllGrad 100 and AllGrad wash solution (Life global, Brussels, Belgium). all components of the upper and lower phase and semen samples were placed in an incubator at 37°C for 20 minutes. 2 mL of the lower phase was transferred into a sterile conical bottom, disposable centrifuge tube, then 2 mL of the upper phase was layered on top of the lower phase using a transfer pipet. 2 mL of liquefied semen gently placed onto the upper phase and Centrifuged for 18 minutes at 350g. Using a transfer pipet, added 2 mL of AllGrad wash and resuspended pellet and Centrifuged for 8 minutes. Again, we removed supernatant from the centrifuge tube using a transfer pipet down to the pellet and resuspend the final pellet in a volume of 0.5 mL AllGrad wash using a 1 mL sterile pipet. Finally, this volume was inseminated by IUI catheter (prince medical, France).

**Sample size**

A table of random numbers was used for randomization. The sample size was calculated based on Aleyasin et al. study[12] (2016) using following formula; at least 49 people were estimated for each group. \(P1=44.6\%, P2=19.6\%, \alpha=0.05\) and \(\beta=0.20\).

\[
n = \frac{(Z_{1-\alpha/2} + Z_{1-\beta})^2 \left[ P_1 (1 - P_1) + P_2 (1 - P_2) \right]}{(P_1 - P_2)^2}
\]
**Statistical methods**

Data were analyzed by descriptive statistics (standard deviation, mean, percent, and frequency), followed by $\chi^2$, T test, mann-whitney. Data were analyzed using statistical software (version 21) (SPSS Inc., Chicago, IL, USA). The significance level for all tests $P < 0.05$ was considered.

**Result**

We assessed 125 patients for eligibility, among them 110 recruited patients randomly divided into two groups (55 patients in each group). Finally, 98 patients completed the follow up (49 patients were placed in control group (IUI), and 49 patients in intervention group (IUI + GCSF). The process of allocating patients is shown in Fig. 1.

Table 1 has shown the socio-demographic and clinical characteristic of the patients, there is no significant difference in appearance between study groups ($P > 0.05$).
Table 1
Clinical characteristics of the patient in study groups. The data show that all groups were comparable (P ≤ 0.05).b

| Groups Variable          | Group control (Letrozole + menotropin + IUI) (n = 49) | Group II (Letrozole + menotropin + IUI + GCSF) (n = 49) | Statistical test | P value |
|--------------------------|------------------------------------------------------|-------------------------------------------------------|------------------|---------|
| Female age*              | 27.22 ± 4.87                                         | 28.89 ± 4.11                                         | T = 1.83         | 0.06    |
| Male age*                | 31.3 ± 4.32                                          | 32.95 ± 5.06                                         | T = 1.73         | 0.08    |
| BMI*                     | 23.87 ± 1.50                                         | 23.69 ± 1.68                                         | T = .56          | 0.57    |
| Type of infertility**    | Primary                                               | 29( 59.2)                                             | X2 = 0.17        | 0.67    |
|                          | Secondary                                             | 20(40.8)                                              |                  |         |
| TSH *                    | 2.16 ± 1.28                                          | 2.49 ± 1.53                                          | T = 0.79         | 0.57    |
| FSH*                     | 4.99 ± 1.71                                          | 5.37 ± 2.28                                          | T = 0.64         | 0.52    |
| LH*                      | 4.35 ± 2.97                                          | 5.28 ± 4.66                                          | T = 0.39         | 0.7     |
| PRL *                    | 4.64 ± 3.28                                          | 4.05 ± 2.18                                          | T = 0.66         | 0.51    |
| Semen analysis           | Sperm concentration                                  | 62.03 ± 30.18                                         |                  |         |
|                          | Progressive motility                                 | 55.23 ± 18.78                                         | T = 1.10         | 0.27    |
|                          | Normal morphology                                    | 45.86 ± 20.41                                         | T = 0.03         | 0.96    |

BMI: body mass index, PRL: Prolactine, LH: luteinizing hormone, FSH: follicle stimulating hormone. TSH: thyroid stimulating hormone

Table 2 shows the biochemical and clinical pregnancy in study groups. The biochemical pregnancy rate was higher in patient who received GCSF (16.3%) in compare to control group (12.2%) but this deference was not significant (P = 0.56). also, GCSF improved the clinical pregnancy rate in intervention group (16.3%) in compare to control group (8.2%), but this was not significant (P = 0.21). In the other word, GCSF could play role in maintaining of pregnancy.
**Table 2**
The comparison of the incidence of pregnancy in study groups. The data show that there is no significant difference between all groups (P ≤ 0.05).

| Groups Variable                      | Group control (Letrozole + menotropin + IUI) (n = 49) | Group II (Letrozole + menotropin + IUI + GCSF) (n = 49) | Statistical test | P value |
|-------------------------------------|-------------------------------------------------------|--------------------------------------------------------|------------------|---------|
| Chemical Pregnancy incidence *      | 6(12.2)                                               | 8(16.3)                                                | X2 = 0.33        | 0.56    |
| Clinical pregnancy incidence *      | 4(8.2)                                                | 8(16.3)                                                | X2 = 0.51        | 0.21    |

* n(%)  

**Discussion**

Based on our knowledge, our study is the first study that evaluated the GCSF effect on patients with unexplained infertility that underwent IUI. The term of unexplained infertility is used for couples with normal semen analysis and the female infertility factor has not been identified[3]. The living fetus and endometrium, as well as successful embryo-endometrium dialog is essential for successful implantation and pregnancy. Although it is endometrial receptivity process is not completely understood, but remodeling of endometrium at the time of window of implantation and local immune shift from adaptive (Type 1 T helper) to innate Type 2 T helper is vital for implantation[13]. A balanced local immune system is required at the time of the window of implantation not only attach the fetus but also regulate the invasion phase. GCSF is a glycoprotein with growth factor and cytokine functions, which are produced in many tissues. Uzmaki and colleagues (1989) identified GCSF receptors in the membranes of human placenta and trophoblast cells and suggested that they probably played an important role in the fetal-placenta unit during human development [14]. Also, GCSF was detected in endometrial cells, follicular cells, syncytotrophoblast, cytotrophoblast, stromal cells and embryonic membranes[15].

In our study, the subcutaneous GCSF injection independently improved implantation and the pregnancy rate in patients with unexplained infertility underwent IUI with normal endometrium, but this was not statistically significant. It seems that the differences will significant if the sample size is increase. Although, our study evaluated the GCSF effect on patients with unexplained infertility that underwent IUI. Whereas, other studies examined the impact of GCSF on patients with repeated implantation failures that are rather similar to our study.

Our findings are in consistent with Scarpelliniet.al. (2012) which reported that GCSF was resulted in higher implantation rates in patients with repeated IVF failures [16], as well as, Aleyasinet.al. (2016), evaluated the effect of systemic administration of 300 µg GCSF by subcutaneous injection in patients with repeated IVF failure. Their findings have shown that GCSF significantly increased the pregnancy rate[12].
Also, Eftekhar and colleagues in an interventional study have shown that although with intra-uterine infusion of GCSF did not improve the endometrial thickness but significantly increased pregnancy rate in women with thin endometrium[17]. Another study which conducted by the Eftekhar and colleagues, their findings have shown that in spite of fewer follicles and the fewer metaphase II oocytes in GCSF group, the implantation and pregnancy rates were more than the control group. They concluded that clinical pregnancies improved by intra-uterine administration of GCSF in oocyte puncture day[18].

In contrast to, Brade and colleagues (2014) examined the effect of GCSF on endometrial thickness and clinical pregnancy rate in patients who underwent IVF treatment and they have reported that GCSF did not improve the endometrial thickness and pregnancy rate[19].

It seems that the difference in the prescribed method, time of administration, age of participants, endometrial thickness and sample size maybe involve in the observed differences in these results. Scarpllini et al.(2012), Aleyasin et.al (2016) studies and our study have a higher sample size, the GCSF was administered systemic via subcutaneous, the study population was younger and had a normal endometrium[16, 12].Whereas, in the conflicting studies, GCSF was administered through intrauterine perfusion and had a smaller sample size and had thin endometrium[17]. Our results showed that GCSF could play an important role in the implantation process and to maintain pregnancy. Salmassi et al. (2005) women underwent IVF treatment and observed that pregnant patients had a continuous increase in serum GCSF levels from day of embryo transfer to day of implantation to day of pregnancy confirmation, but the patients who were not pregnant, they showed a little increase in GCSF level, then its level significantly reduced by failure of implantation. These authors concluded that this cytokine played an important role in the pregnancy and maintained of pregnancy[20]. Also, Rahmati and colleagues reported that infertile women with implantation failure had very low amounts of GCSF receptor in maternal-fetal interface. The interesting point was that stimulation with high dose of GCSF was able to increase expression of GCSF receptors in these patients[21]. GCSF can affect reproductive, implantation and pregnancy through several mechanisms: GCSF induces the proliferation and invasion of trophoblast in pregnancy[22]. GCSF also plays a key role in embryo implantation process through the regulation of fundamental genes which responsible for the embryo attachment, cell migration, tissue remodeling and angiogenesis. These events are inevitable for a successful implantation and placentation[21]. Finally, GCSF is involved in adaptation changes that induce immune tolerance in pregnancy. Pregnancy is an immune challenge for the mother. GCSF shifts the T cell cytokine profile to TH2 responses and enhance the T regulatory cells producing IL10 and differentiation of the tolerant dendritic cells [23]. These are important parts of immune regulation that occur before and after implantation in the uterus[15].

The main strengths of our study is randomized controlled trial and the first evaluation of GCSF effect on fertility in patients with unexplained infertility whom treated by IUI. Other strengths of this study evaluated systemic administration and the dose of GCSF, which is easier, more tolerable, and more economical than repeatative doses or local infusion. However, this study has some limitations which should be considered in the interpretation of the results because of the nature of study, unblinded study was not used. In
addition, we have not studied pregnancy results in two groups that could help to better interpret the results and safety of the GCSF administration.

Conclusion

Systemic administration of 300 µg GCSF in the two days after IUI slightly improves the clinical pregnancy rate in patients with unexplained infertility treated with IUI. Nevertheless, our findings do not support routine use of GCSF in unexplained infertility women with normal endometrial thickness.

Abbreviations

GCSF
Granulocyte Colony Stimulating Factor
IUI
Intra Uterine Insemination

Declarations

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Availability of data and materials

The primary data for this study from the authors on direct request

Authors' Contribution

SA and ST contributed in Conception and design, data acquisition, writing and confirming the final draft. 2. MA, FN, PG contributed in data acquisition, Recording the outcomes and providing resources. All authors approved the final version for submission. SA supervised the study.

Ethical approval and consent to participate
The study was followed the Declaration of Helsinki Guideline and approved by the ethics committee of Yasuj university of medical sciences, Yasuj, Iran (www.irct.ir: IRCT20160524028038N4). Written consent was obtained from all participates.

**Competing interest**

The authors have no conflict of interest in this manuscript.

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Figures
Figure 1

The process of allocating patients.