REVIEW ARTICLE

Role of granulocyte colony-stimulating factor in human reproduction

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As new research reveals, granulocyte colony-stimulating factor (G-CSF) plays an effective role in pregnancy success, considering that it not only affects the embryo implantation and ovarian function but also it promotes endometrial thickening and improves the pathophysiology of endometriosis, which all fundamentally lead to reducing pregnancy loss. In this review, we focus on the role of G-CSF in human reproduction. We summarized its role in ovulation, luteinized unruptured follicle syndrome, poor responders, improving repeated *in vitro* fertilization failure, endometrial receptivity and treatment of thin endometrium, and recurrent spontaneous abortion.

Key words: Fertilization *in vitro*, granulocyte colony-stimulating factor, habitual abortion, ovulation

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INTRODUCTION

Some cells such as fibroblasts, monocytes, macrophages, endothelial cells, stromal cells, and bone marrow cells make a special cytokine called granulocyte colony-stimulating factor (G-CSF).[1] G-CSF has some functions; however, its major role is to stimulate the neutrophils proliferation and their differentiation in the bone marrow. Besides, their release to the bloodstream is under control of G-CSF. Actually, in mature neutrophils, phagocytosis and oxidative process are boosted by G-CSF.[2]

In 1983, G-CSF was found in mice for the first time and later in 1986, and it was purified in human G-CSF (hG-CSF).[3] hG-CSF has a specific receptor (G-CSF receptor) which is located on the surface of some cells such as myeloid progenitor cells, myeloid leukemia cells, mature neutrophils, platelets, monocytes, lymphoid cells, some T-cells, and B-cells.[4] Even several nonhemato cells, for example, endothelial cells, placenta cells, trophoblastic cells, the fetomaternal interface, and granulose luteinized cells include such a receptor that h G-CSF's activities are mediated through it.[5]

As new research reveals, G-CSF plays an effective role in pregnancy success, considering that it not only affects the embryo implantation and ovarian function but also it promotes endometrial thickening and improves the pathophysiology of endometriosis, which all fundamentally lead to reducing pregnancy loss.[6-8] In fact, since G-CSF improves implantation, it is presented as an essential item for implantation and even it is a remedy for implantation failure.[9]

In general, human decidual macrophages, ovulation, and ovarian function are impacted by G-CSF. Furthermore, it influences granulosa cell functions (granulocyte-macrophage-CSF [GM-CSF]), as well as, improving ovarian stimulation in poor responders, and even it is predictive of *in vitro* fertilization (IVF) outcome. In addition, it is considered as a biomarker for oocytes/embryos that are potentially

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able to implant. Even the number of unexpected repeated pregnancy loss reduces by G-CSF. Furthermore, G-CSF is known as a key factor in the genesis of early endometriotic lesions and autoimmunity suppressor.[6] Furthermore, it was claimed that serum G-CSF was kept at the high level during pregnancy. Fetal chorionic villous and maternal decidual tissues release G-CSF in the first trimester and during it.[10]

In this review, we focus on the role of G-CSF in human reproduction. We summarized its role in ovulation, luteinized unruptured follicle (LUF) syndrome, poor responders, improving repeated IVF failure, endometrial receptivity, and recurrent spontaneous abortion.

GRANULOCYTE COLONY-STIMULATING FACTOR AND ITS ROLE IN OVULATION

In women with normal menstrual cycles, G-CSF gradually boosts during the follicular phase and reaches its peak in ovulation time. G-CSF leads to leukocyte accumulation in the follicle, follicular wall and accelerates ovulation. [11] Besides, during ovarian stimulation, parallel to follicular growth, an important increase will be noticed in serum concentration of G-CSF and the number of white blood cells.[12] G-CSF concentration had been directly correlated the quality of oocyte, and also it was related to the patient's age. In all types of assisted reproductive technology (ART), the number of retrieved oocyte and quality of them is absolutely vital for the success of the process.[13]

THE ROLE OF GRANULOCYTE COLONY-STIMULATING FACTOR IN LUTEINIZED UNRUPTURED FOLLICLE SYNDROME

LUF syndrome is considered as an intractable ovulation disorder that is usually noticed during the cycle of ovulation induction.[14] It had been shown by Shibata *et al*. that additional use of G-CSF significantly prevented LUF syndrome during ovulation induction ($P = 0.013$). Since no sever adverse effect by G-CSF administration was observed, it was concluded that G-CSF can be used in LUF syndrome.[14]

THE ROLE OF GRANULOCYTE COLONY-STIMULATING FACTOR IN FOLLICULAR FLUID

Recently, the potential role of serum and follicular fluid (FF) G-CSF, which is a noninvasive biomarker of oocyte competence and embryo choice IVF cycles, is being suggested. In fact, G-CSF level in both serum and FF is the main predictor of IVF outcome.[15,16]

Lédée *et al*. and others demonstrated that, in subsequent implantation, the follicular G-CSF was highly predictive. Their results showed that the follicular level of G-CST had higher discriminatory power than embryo morphology for prediction of ongoing pregnancy (0.77 [0.69–0.83], $P = 0.001$ vs. 0.66 [0.58–0.73], multivariate logistic regression analysis).[17] In contrast, a study by Kahyaoglu *et al*. confirmed that levels of G-CSF concentration in both serum and follicular microenvironment in polycystic ovary syndrome women have no correlation with good ovarian response or clinical pregnancy rates.[11]

ROLE OF GRANULOCYTE COLONY-STIMULATING FACTOR IN OOCYTE MATURATION

In early 2005, it was suspected that there was a positive link between G-CSF concentration in FF and IVF outcomes.[18] The positive role of the high concentrations of G-CSF in the probability of implantation and subsequent pregnancy was proved by follow-up studies.[19] Such a correlation is just applied to G-CSF and no other growth factors and cytokines.[19] A recent publication demonstrated that oocytes from follicles with a concentration of >30 pg/ml G-CSF show the highest probability; however, oocytes with a concentration of 18.4–30 pg/ml showed mid-range probability and those with a concentration of 18.4 pg/ml presented low probability of pregnancy in an ART.[17]

Actually, systemic administration of G-CSF in the follicular phase would be suggested because of a great correlation between high G-CSF concentration in FF and desirable pregnancy prognosis in ART. An improvement in the pregnancy rate has been indicated by considering the initial results. Evidently, an important reduction in LUF syndrome in clomiphene citrate cycles will be noticed by a single does of recombinant hG-CSF (Lenograstim, 100 mg) 48 h before the administration of human chorionic gonadotropin. Apparently, this way 90% of all LUF syndromes will be avoided.[20]

Among the embryos which are generated after IVF/ICSI, FF G-CSF quantification can be considered as a new tool to go for the highest potential rate of pregnancy. The effectiveness of embryo selection might be improved by combining the FF G-CSF concentration with the morphology scale. Quantification of follicular G-CSF will probably increase the ongoing pregnancy rate throughout some ways such as providing better choices of embryos, limiting multiple pregnancies, decreasing embryo cryostorage, and applying some recently destroyed embryos.[17] Before fertilization, the follicular concentration of G-CSF can be used as a good biomarker of oocyte competence.[21]

ROLE OF GRANULOCYTE COLONY-STIMULATING FACTOR IN CULTURE MEDIUM

By adding recombinant GM-CSF to the culture medium, not only human embryos can be closer to *in vivo* condition

but also the efficacy of ART cycles improves. A culture medium named embryogenic is now commercially accessible. Furthermore, for culture medium with additional G-CSF (0.5 ng/ml), a patent is requested. A significant increase was showed by Tevkins *et al.* in both implantation rate and progressive clinical pregnancy rate in embryos cultured, in a culture medium named EmbryoGen medium compared to the standard combination of medium (ISM1 + VA) (20.4% and 17.4% vs. 11.6% and 9.1%).[21]

Besides, it was demonstrated by Ziebe *et al.* t hat not only adding GM-CSF to embryo culture medium enhances survival of transferred embryos to 12 weeks but also it has a protective effect on culture-induced embryo stress. In women, suffering from previous miscarriage GM-CSF may be particularly efficacious.[22]

In addition, adding G-CSF to bovine embryo culture has some other benefits including increasing development and posttransfer survival as well as decreasing pregnancy loss.[22] Actually, IVF pregnancy chance was increased significantly using endometrial coculture, G-CSF >130 pg/ml.[23]

ROLE OF GRANULOCYTE COLONY-STIMULATING FACTOR IN POOR RESPONDERS

Almost 9%–18% of ART cycles are poorly responded to ovarian stimulation happenings.[24] Fortunately, in poor responders, the number of retrieved oocytes and pregnancy rates were improved by G-CSF adjuvant therapy comparing to the ones were in the preceding cycles. Takasaki *et al*. presented G-CSF adjuvant therapy could be a useful therapeutic tool to increase fertility in chosen poor responders.[25]

G-CSF has been administrated as a supplementation in women with low responders in ART cycles to develop the response of the ovary to the pharmacologic stimulatory treatment. Based on a randomized controlled study in women undergoing ART, it was demonstrated that G-CSF supplementation is effective to improve the results in the ART treatment of low responder women.[8] This cytokine might have a local paracrine effect on oocytes and enhance their abilities to improve under pharmacological stimulation and helping to be fertilized.[13]

ROLE OF GRANULOCYTE COLONY-STIMULATING FACTOR IN CURING NORMAL INFERTILE WOMEN

The implantation rates still remain literally low although some main developments have been discovered in assisted reproductive techniques. To have a successful implantation, three major items are needed:

1. Good quality embryo
2. Receptive endometrium
3. Good embryo transfer technique.[26]

According to a randomized, parallel, double-blinded, placebo-controlled clinical trial that was done by Barad *et al.* in normal IVF patients, 73 patients received G-CSF and 68 other patients received placebo. It was presented that endometrial thickness which was highly enhanced by about 1.36 mm and G-CSF had no effects on both clinical pregnancy and implantation rates, as well as endometrial thickness, implantation rate, or clinical pregnancy rates.[27]

To assess the G-CSF effects on IVF outcomes in normal women, eftekhar *et al.* proposed a study on infertile women with normal endometrial thickness in two groups. In the G-CSF group (n = 50), 300 μg transcervical intrauterine of G-CSF was administered at the oocyte retrieval day and the controls (n = 50) were treated with the standard protocol. Chemical, clinical, and ongoing pregnancy rates, implantation rate, and miscarriage rate were compared between groups and they were showed in normal IVF patients with a normal endometrial thickness that the intrauterine infusion of G-CSF did not improve pregnancy outcome.[26]

ROLE OF GRANULOCYTE COLONY-STIMULATING FACTOR IN IMPROVING REPEATED *IN VITRO* FERTILIZATION FAILURE

The definition of repeated implantation failure (RIF) is a failure of implantation in at least three repeated IVF cycles that one to two high-quality embryos transferred in each one. Approximately, 40% of IVF cycles will fail during one cycle of ART. IVF failure has some reasons such as early stage of implantation failure or early abortion. Main causes of failures are due to embryo quality and implantation failure.[28] Some significant roles have been found for G-CSF in both embryo implantation process and maintenance of pregnancy. Moreover, some promising results have been showed using local intrauterine infusion of G-CSF in patients undergoing IVF.[7,8,29]

A multicenter, randomized, controlled trial which was done by Aleyasin *et al*. indicated that implantation and pregnancy rates in infertile women with repeated IVF failure can be significantly boosted by the administration of single-dose systemic subcutaneous G-CSF before implantation is done.[30]

In 2012, a randomized controlled study on 109 recurrent implantation failure (RIF) patients, a daily dose of 60 mg G-CSF was started and continued to be received for a further 40 days after a positive pregnancy test. They reported 43.1% the clinical pregnancy rate per embryo transfer in the G-CSF group and 21.6% in the placebo group (Saline

injection), which resulted in a highly significant difference. No undesirable side effects were reported. Furthermore, the pregnancy rates for both day 2 and day 5 embryo transfers were highly different.[31]

Eftekhar *et al*. evaluated the efficacy of transvaginal perfusion of G-CSF on repeated implantation failure. G-CSF was administrated in the intervention group by intrauterine infusion, which led to a highly significant improvement in the pregnancy outcome in this group.[9]

ROLE OF GRANULOCYTE COLONY-STIMULATING FACTOR IN ENDOMETRIAL RECEPTIVITY

G-CSF is necessary for many items such as mobilization and recruitment of lymphocytes, uterine dendritic cells, and macrophages. G-CSF plays a vital role as an endometrial remodeling factor for implantation window. G-CSF prepares endometrium in only a few days for some reasons such as suppressing any immune aggression to present an embryo and allow sufficient endometrial receptivity.[32] Successful implantation needs some items including a good quality embryo, a receptive endometrium, and a good embryo transfer method. The normal thickness of the endometrium, which is between 7 and 14 mm in the secretary phase, is a key element for a successful pregnancy in IVF cycles.[26] Shapiro *et al*. showed that if endometrium thickness is less than 6mm, pregnancy will not happen.[33] Al-Ghamdi *et al*. concluded that thin endometrium is associated with more miscarriage.[34] A wide variety of treatments has been used to enhance endometrium thickness such as extended estradiol, low-does aspirin, vaginal treatment by vitamin E, L-arginine and sildenafil citrate, intrauterine administration of bone marrow stem cells, and progenitor cells.[35]

Kunicki *et al*. showed that endometrium thickness in the patient with extremely thin endometrium can be increased using G-CSF when other methods failed to improve it. Improving endometrial thickness after administering of G-CSF will probably increase the chance of pregnancy.[36]

Tehraninejad *et al*. understood that infusion of G-CSF in the endometrial cavity is a safe and effective method for increasing endometrial thickness in patients with thin and unresponsive endometrium.[16]

Apparently, chronically unresponsive thin endometrium, which was resistant to traditional remedies, such as increased E2 support and sildenafil could be affected positively by endometrial perfusion with G-CSF. An increased spurt in endometrial thickness can be noticed during 48–72 h of G-CSF administration.[6]

For frozen embryo transfer (FET) cycles, the endometrium is consistently prepared with estrogen and progesterone supplementation.[37] Gleicher *et al*. showed that G-CSF is a beneficial remedy in FET cycles for patients suffering from the unresponsive, inadequate, and thin endometrium.[38]

Eftekhar *et al*. failed to demonstrate that G-CSF potentially improves endometrial thickness; however, it was claimed that it will possibly improve chemical and clinical pregnancy rate of the infertile women suffering from thin endometrium in FET cycle.[39]

Li *et al*. failed to indicate the potential role of G-CSF in improving embryo implantation and clinical pregnancy rate of the infertile women with thin endometrium.[10]

Mishra *et al*. showed that G-CSF led to a very small increase in endometrial thickness in women dealing with persistent thin endometrium although no improvement in their pregnancy rates was noticed.[40]

GRANULOCYTE COLONY-STIMULATING FACTOR AND ITS ROLE IN RECURRENT SPONTANEOUS ABORTION

Recurrent miscarriage's definition (RM) is the occurrence of three or more clinically detectable pregnancy losses in the first trimester. RM is as frequent as 1% in women with reproductive age. In general, the recognized causes of RM are many. Take the example of parental chromosomal defects, mainly reciprocal or Robertsonian translocations, infections, endocrinological cause (thyroid disease, diabetes, and polycystic ovaries), uterine abnormalities, antiphospholipid antibody syndrome, and other autoimmune conditions.[41]

Surprisingly, over 40% of RM cases remain unexplained. However, for such cases, possible causes seem to be immune dysfunction or alloimmune response.

RM could be caused due to an imbalance in the Th1/Th2 system. The great part is that, during pregnancy in the uterine tissues of Th1 cytokine production, they act as a cytotoxic one instead of Th2 cytokine production and have an immune suppression role. The negative parts are about rejecting the embryonic allograft.[8]

Scarpellini and Sbracia use recombinant G-CSF (RG-CSF) as a way of treatment for couples with RM. Sixty-eight women with RM of unknown cause and being treated with intravenous human immunoglobulin were accidentally chosen to be treated by either Rg-CSF or placebo. Women in the treated group received a dose of 1 g (100,000 IU) kg/day of Filgrastim (Neupogen, Dompe, Italy) starting from the 6th day of ovulation until the end of menstruation or

even the end of the 9th week of pregnancy. Another group named placebo group contained 33 women receiving saline exactly the same administration and period of time, the way treated group had received. It was detected that, in G-CSF group, 29 out of 35 (82.8%) women delivered a healthy infant, whereas, in the placebo group, live birth rate was 48.5% (16 out of 33) ($P = 0.0061$).[8]

Santjohanser *et al*. performed a retrospective cohort study in women with RM undergoing ART. One hundred and twenty-seven women (199 cycles) with RM (at least 2 early miscarriages), 49 (72 cycles) receiving G-CSF, and 78 (127 cycles) who were controls received either no medication (subgroup 1) or cortisone, intravenous immunoglobulin, or low molecular weight heparin (subgroup 2) undergoing ART for IVF/intracytoplasmic sperm injection were analyzed. They showed that the number of early miscarriages was significantly higher in the G-CSF group as compared to the subgroups (G-CSF 2.67 ± 1.27, subgroup 1: 0.85 ± 0.91, subgroup 2: 0.64 ± 0.74).[42]

WHAT ARE GRANULOCYTE COLONY-STIMULATING FACTOR SIDE EFFECTS?

Bone pain, general fatigue, headaches, insomnia, anorexia, nausea, and/or vomiting are considered as some side effects of treatments with G-CSF. Moreover, dyspnea, chest pain, hypoxemia, diaphoresis, anaphylaxis, syncope, and flushing are some other it's adverse effects.[43]

CONCLUSION

Oocyte maturation, endometrial receptivity, development of preimplantation embryos, and trophoblast invasion are affected and promoted by G-CSF. Evidently, it increases the pregnancy rate in ART treatment, especially in patients who had RIF and it decreases the abortion rate in patients with RSA, and even it reduces preterm birth of preeclampsia.

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Conflicts of interest
There are no conflicts of interest.

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