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# Clinical Applications of Recombinant Human Granulocyte Colony-Stimulating Factor in Reproductive Medicine: Expert Consensus

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**Abstract:** Recombinant human granulocyte colony-stimulating factor (rhG-CSF) is primarily indicated for neutropenia caused by various etiologies in clinical practice. Recently, it has been shown to have some therapeutic effects and be safe for mother and child in some clinical research and treatment of reproductive diseases. However, until now, there is currently no authoritative expert consensus and guidelines published. To standardize the research and rational application of rhG-CSF in reproductive medicine-related fields, the Committee on Reproductive Immunity of the China Association for Promotion of Health Science and Technology (the Committee) organized experts from relevant disciplines to formulate the Expert Consensus, which is based on the latest international basic study, clinical research evidence, and the national situation of China. According to evidence-based data, recommendations were established on the applicable population, medication methods, and timing of discontinuation of rhG-CSF in reproductive medicine. It is recommended that rhG-CSF could be applied in patients with thin endometrium, which was ineffective to standard treatments, in unexplained recurrent spontaneous abortion (RSA), in unexplained recurrent implantation failure (RIF), and could attempt to be applied in unexplained RSA with the slow rise of human chorionic gonadotropin and embryo growth retardation. After the patient signs the super instruction for medication and treatment informed consent form, rhG-CSF can only be used for patients with thin endometrium, unexplained recurrent miscarriage, unexplained repeated embryo implantation failure, slow rise of human chorionic gonadotropin. Comprehensive communication between gynecologists and obstetricians and patients before therapy is essential according to that the rhG-CSF treatment would be inefficient when a genetic abnormality, embryo abnormality, heterotopic pregnancy and other unknown disorder exist.

**Keywords:** Recombinant human granulocyte colony-stimulating factor; Reproductive medicine; Thin endometrium; Recurrent spontaneous abortion; Recurrent implantation failure.

## Introduction

In recent years, recombinant human granulocyte colony- stimulating factor (rhG-CSF) has shown impressive progress in the treatment of patients with thin endometrium or recurrent implantation failure (RIF) who have failed to respond to conventional treatment. However, there is still limited understanding of the new developments, mechanism of action, usage, and side effects of rhG-CSF in the field of reproductive medicine. The use of rhG-CSF in reproductive medicine is at two extremes: some gynecologists and obstetricians (OB/GYN) do not know how to use it and are hesitant to use it, while others use it excessively for patients without clear indications.

To standardize the clinical application of rhG-CSF in reproductive medicine, a consensus writing group composed of experts in obstetrics and gynecology, reproductive medicine, rheumatology and immunology, and pharmacology was organized by this Committee. Based on domestic and international research progress and combined with the actual situation in China, the expert consensus was developed through joint discussion. The consensus focuses on the application of rhG-CSF in thin endometrium, unexplained recurrent implantation failure (URIF), unexplained recurrent spontaneous abortion (URSA), slow rise of human chorionic gonadotropin (hCG), and delayed embryo development, providing objective evaluation and guidance on the applicable population, contraindications, medication methods, relevant examinations before and after medication, medication monitoring, observation and management of adverse reactions to drugs, and maternal and infant safety during medication in pregnancy.

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## 1. Clinical applications of rhG-CSF in reproductive medicine

### 1.1. Application of rhG-CSF in patients with thin endometrium

The research has shown that thin endometrium can lead to a decreased pregnancy rate[1,2] and that appropriate endometrial thick-

ness and morphology are crucial for successful pregnancy[3,4]. However, there is still controversy over the boundary between a "thin" and "normal" endometrium, with most researchers considering a minimum thickness of 7.0 mm or more to be beneficial for achieving the best pregnancy rate[1,2]. Therefore, currently in clinical practice, a thickness below 7.0 mm is commonly used as the criterion for diagnosing a thin endometrium. Conventional methods for treating a thin endometrium include high-dose estrogen, low-dose aspirin, and traditional Chinese medicine, among others. In addition to the above standard treatments, rhG-CSF, sildenafil, pentoxifylline, vitamin E, L-Arginine and platelet-rich plasma have been attempted recently. Endometrial perfusion of rhG-CSF is effective in the treatment of thin endometrium, which was resistant to traditional remedies. At the same time, it has the advantages of low price and convenient use, which promote its clinical application.

At present, the basic research and clinical evidence regarding the effectiveness of rhG-CSF in thin endometrial patients mainly includes: basic research confirms that intrauterine infusion of rhG-CSF can affect endometrial vascular remodeling, improve endometrial blood flow and thickness[5] , The rhG-CSF can increase the expression of vascular endothelial growth factor (VEGF) in the endometrium, increase the expression of integrin αvβ3 and leukemia inhibitory factor (LIF)[6]. And also promote immune tolerance at the maternal-fetal interface by locally regulating the immune system of the nourishing layer and decidua[7]. For thin endometrial patients undergoing in vitro fertilization and embryo transplantation (IVF-ET) or intracytoplasmic sperm injection (ICSI), whether fresh or frozen embryo transfer, intrauterine infusion of rhG-CSF can increase endometrial thickness, improve endometrial blood flow, and increase clinical pregnancy rates[5,8–14]. For patients with recurrent spontaneous abortion (RSA), intrauterine infusion of rhG-CSF can also improve endometrial receptivity and reduce the miscarriage rate[15,16].

**[Expert Opinions and Recommendations]**

Patients eligible for intrauterine infusion of rhG-CSF with thin endometrium: Following individualized comprehensive etiological screening and analysis, for patients with thin endometrial lining who are unresponsive to conventional estrogen, aspirin, and traditional Chinese medicine treatments, uterine cavity infusion of rhG-CSF can be attempted to improve endometrial receptivity.

1.1.1 Recommendations for the clinical application of intrauterine perfusion of rhG-CSF for patients using different assisted reproduction and transplantation methods are as follows:

1.1.1.1 For patients undergoing fresh cycle embryo transplantation, intrauterine perfusion of rhG-CSF 300 μg can be performed on the day of hCG[9].

1.1.1.2 For patients undergoing frozen-thawed embryo transplantation and using hormone replacement therapy to prepare the endometrium, intrauterine, perfusion of rhG-CSF can be performed on the 8th, 11th, and 13th days of the menstrual cycle, with 300 μg each time.

1.1.1.3 For patients undergoing natural conception, rhG-CSF intrauterine infusion treatment can be performed for three consecutive days during the periovulatory period (2 days before ovulation, 1 day before ovulation, and the day of ovulation) for 2 menstrual cycles, or rhG-CSF intrauterine infusion can be started 2–3 days after menstruation and administered once every 2–3 days for a total of 3–4 times[15].

### 1.2. Application of rhG-CSF in unexplained RIF

RIF refers to the failure to achieve clinical pregnancy in women under 40 years of age who have undergone at least 3 fresh or frozen embryo transfers, with at least 4 high-quality embryos transferred[17]. The cause of RIF may involve maternal factors such as reproductive organ disorders, poor endometrial receptivity, thrombotic disorders, autoimmune diseases, and embryo factors such as chromosomal abnormalities, zona hardening, poor embryo culture environment, and suboptimal transplantation techniques[18].

In recent years, many researchers have proposed treatments for maternal factors of RIF, including hysteroscopic surgery to correct abnormal uterine anatomy[19]; endometrial scratching[20,21]; uterine cavity infusion of hCG[22], rhG-CSF[23,24] or autologous fresh peripheral blood mononuclear cells (PBMC)[25,26]; contraction inhibitors[27], anticoagulants[28–30]; and immunotherapy[31,32].

Unexplained RIF (URIF) is defined as the RIF for which etiology remains unknown despite comprehensive screening for genetic, endocrine, infectious, immunological, and male factors, as well as RIF with poor response to corresponding conventional treat- ments[13,33]. Multiple studies have suggested that in the treatment of URIF, the use of uterine cavity irrigation and subcutaneous injection of rhG-CSF can improve chemical pregnancy rates[12,23,24,34], implantation rates, and clinical pregnancy rates[13,35,36] and no related adverse events have been reported[37]. Therefore, it is inferred that rhG-CSF uterine cavity irrigation and subcutaneous injection are promising methods for the treatment of URIF[12,13,18].

**[Expert Opinions and Recommendations]**

1.2.1 Applicable people for rhG-CSF in treatment of RIF: The rhG-CSF is recommended as an alternative treatment for RIF of unknown etiology after personalized screening and analysis of the underlying causes.

1.2.2 Recommendations for the clinical application of rhG-CSF in the treatment of URIF:

1.2.2.1 For patients undergoing fresh embryo transfer, a uterine infusion of rhG-CSF at 300 μg on hCG day is recommended, followed by daily or every other day subcutaneous injections of rhG-CSF at 100–200 μg (2–4 μg/kg/dose) until 15 days after oocyte retrieval or until normal embryo development is achieved[36].

1.2.2.2 For patients undergoing frozen-thawed embryo transfer, subcutaneous injections of rhG-CSF at 100–200 μg (2–4 μg/kg/dose) are recommended on the day of transplantation and continued daily or every other day until normal embryo development is achieved.

### 1.3. Application of rhG-CSF in URSA

RSA has complex etiology, among which genetic factors, abnormal maternal reproductive tract anatomy, endocrine factors, thrombophilia factors (including inherited and acquired thrombophilia), and maternal immune factors (including autoimmune and alloimmune) are the most common causes. It has been reported that even with comprehensive screening, 50% of RSA patients have unknown reasons. With the deepening of RSA etiology research and increasing examination projects, the proportion of unexplained recurrent spontaneous abortion (URSA) has significantly decreased. An analysis of 1676 Japanese RSA patients found that 69% of RSA patients had no clear cause without embryo chromosomal testing, while the incidence of URSA de-

creased to 25% after embryo chromosomal testing[38]. Currently, there is no standard treatment plan for URSA at home and abroad. Recently, reported treatment options at home and abroad include lymphocyte immunotherapy, intravenous immunoglobulin, tumor necrosis factor α antagonists, hydroxychloroquine, cyclosporine, and rhG-CSF treatment of the therapeutic regimens reported at home and abroad recently include lymphocyte immunotherapy, intravenous immunoglobulin, tumor necrosis factor α antagonists, hydroxychloroquine, cyclosporine and rhG-CSF in women with URSA.

An increasing number of studies suggest that rhG-CSF is a promising method for treating URSA[13], as it can improve pregnancy and live birth rates while reducing miscarriage rates[39,40]. Research indicates that rhG-CSF may work by activating the invasive ability of the endometrial tissue, promoting endometrial cell growth, and aiding in embryo implantation.

**[Expert Opinions and Recommendations]**

1.3.1 Applicable people for rhG-CSF in treatment of RSA: After individualized comprehensive etiological screening and analysis, rhG-CSF is recommended as a possible treatment option for URSA, but not recommended as a routine treatment method for RSA without comprehensive etiological screening.

1.3.2 Recommendations for the clinical application in rhG-CSF treatment of URSA: Starting from the ovulation day or embryo transfer day, administer subcutaneous injection of rhG-CSF 100–200 μg (2–4 μg/kg/time) daily or every other day until menstruation or until the 7–8$^{th}$ week of confirmed pregnancy when fetal heartbeat is detected by vaginal ultrasound, then discontinue the medication as appropriate.

### 1.4. Application of rhG-CSF in slow rising hCG and embryo growth retardation

Research has shown that rhG-CSF has a promoting effect on the growth and invasion ability of trophoblast cells. The hCG level of RSA patients after rhG-CSF application is significantly higher than that of the control group[39]. For patients with low initial hCG levels and repeated biochemical pregnancies, the use of rhG-CSF can improve clinical pregnancy rates and live birth rates[18].Patients with immune function abnormalities with increased NK cell ratio or TNF-α/IL-10 ratio may be a dominant population[41]. Currently, it is believed that rhG-CSF may play a role in regulating endometrial receptivity, regulating the biological activity of trophoblast cells, and regulating the immune cell function at the maternal-fetal interface. For patients with slow hCG rise and delayed embryonic development, rhG-CSF can provide some benefits. However, large-scale, high-quality clinical studies are still needed to verify its efficacy and safety. In the absence of contraindications, rhG-CSF can be used to increase clinical pregnancy rates.

**[Expert Opinions and Recommendations]**

1.4.1 Applicable people for rhG-CSF in treatment of slow rise of hCG levels and embryo growth retardation: After excluding contraindications and obtaining informed consent from patients, the application of rhG-CSF in the treatment of slow rise of hCG or/and embryonic growth retardation can be attempted to improve the success rate of fetal preservation.

1.4.2 The recommended dosage is daily or alternate-day subcutaneous injections of rhG-CSF at a dose of 100–200 μg (2–4 μg/kg per injection) until hCG levels or embryo development return to normal. Continue the treatment of another week when hCG and ultra sound examinations shows no abnormalities.

## 2. Maternal and fetus safety evaluation of rhG-CSF administration

The safety of medication use has always been an important concern for clinical doctors during pregnancy. There is almost no evidence to show that the use of rhG-CSF during pregnancy carries any safety risks. Animal model data shows that placental thrombosis only occurs when the dose in rabbits is 1000 times higher than the human dose. Studies have reported that in patients receiving rhG-CSF treatment (during implantation and early pregnancy), no significant adverse reactions were observed in the mother, fetus, or infant, only mild adverse reactions were observed[42]. In addition, the Severe Chronic Neutropenia International Registry (SCNIR) has a large amount of data on the use of rhG-CSF during pregnancy, and no increase in incidence or mortality rates has been observed[42,43]. One study showed that pregnant women with chronic neutropenia who received long-term rhG-CSF treatment did not have any adverse effects on pregnancy or the fetus. Therefore, it is recommended that women with chronic neutropenia use rhG-CSF appropriately during pregnancy[44]. Another study showed that the use of rhG-CSF in assisted reproductive therapy did not increase the risk of adverse perinatal outcomes[45,46]. Meta-analysis shows that the use of G-CSF in pregnant women is safe[47] effective, and tolerable. In special cases, rhG-CSF can be used for peripheral blood stem cell transplantation and bone marrow transplantation with pregnant women as donors, and no adverse effects on the fetus have been found[48]. The study also found that rhG-CSF can pass through the placenta and improve the survival rate of immature embryos. It reduces the occurrence of miscarriage by promoting the growth of trophoblast cells and the metabolism of the placenta, and stimulating the production of fetal granulocytes. Most of the safety data on the use of rhG-CSF is obtained from statistical data on neutropenic patients receiving rhG-CSF treatment or healthy bone marrow stems cell donors, who use rhG-CSF doses 3–10 times higher than those used in reproductive medicine, so it is considered safe to use low-dose rhG-CSF in patients with adverse pregnancies.

**[Expert Opinions and Recommendations]**

Although there are no reported studies on the adverse effects of using rhG-CSF during pregnancy on pregnant women and newborns, rhG-CSF is a potent cytokine that promotes neutrophil proliferation and is not a routine medication for pregnant women. Therefore, the clinical application of rhG-CSF should be strictly regulated, and unnecessary, high-dose, or prolonged use should be avoided.

## 3. Pre-dosing examination, medication monitoring and operation methods of rhG-CSF

### 3.1. Examination and monitoring before intrauterine infusion of rhG-CSF

Before performing intrauterine infusion of rhG-CSF, it is neces-

sary to exclude reproductive tract infections and pregnancy by conducting examinations such as vaginal secretions and blood routine. It is necessary to exclude reproductive tract infections and pregnancy by conducting examinations such as vaginal secretions and blood routines. During the assisted reproductive period, transvaginal color Doppler dynamic monitoring of endometrial thickness, morphology, and follicular development should be carried out.

### 3.2. Operation method

The drug was aspirated with a 2 mL syringe and connected to a disposable artificial insemination tube, which was injected through the cervical canal into the uterine cavity. After infusion, maintain a head-down and buttock-up position for 10−15 minutes. Monitor changes in endometrial thickness, morphology, and uterine blood flow dynamics at 48 and 96 hours after infusion[33]. If the endometrium remains less than 7 mm, repeat the uterine infusion.

## 4. Common adverse effects and treatment of rhG-CSF

Common adverse effects of rhG-CSF subcutaneous injection include skeletal muscle aches, loss of appetite and fatigue. A few patients may experience fever, redness and swelling at the injection site, etc., which are generally well tolerated without special treatment.

For patients using rhG-CSF for the first time, safety monitoring should be strengthened. Pharmacokinetic data show that after a single subcutaneous injection, the peripheral blood neutrophil count (ANC) reaches its maximum value 12 hours after administration[49,50]. In healthy individuals, rhG-CSF 150 μg is administered daily at a dose of 150 μg subcutaneously for 10 consecutive days, maintaining an ANC of 10–20 × $10^9$/L. After stopping the medication, the ANC rapidly decreases within 1–2 days[51], and the peripheral blood white blood cells (WBC) return to normal levels within 4 days[52].

**[Expert Opinions and Recommendations]**

According to the patient's condition, subcutaneous injection of rhG-CSF 100−200 μg (2–4 μg/kg/time) is administered. Blood routine examination would be performed before each injection. If WBC ≤ 25 × $10^9$/L, the medication can continue; if WBC ≥ 25 × $10^9$/L, the injection can be postponed until WBC ≤ 25 × $10^9$/L. If WBC ≥ 25 × $10^9$/L is not found in three blood routine monitoring, the frequency of monitoring can be adjusted to once every 6–10 days. During medication, drink plenty of water (more than 2 L per day) to promote drug metabolism.

## 5. Consensus statement

**5.1** This consensus serves only as a guiding suggestion for some issues that rhG-CSF faces in the field of reproductive medicine. It cannot replace the independent professional judgment of clinical physicians based on the patient's condition, nor can it deny the research conclusions obtained by the medical team based on their research situation. This consensus puts forward opinions and suggestions for the rational application and research of rhG-CSF, it cannot replace the independent professional judgment of clinical physicians based on the patient's condition, nor can it deny the research conclusions obtained by the medical team based on their research situation.

**5.2** This consensus provides opinions and suggestions for the rational application and study of rhG-CSF, but there are some shortcomings and deficiencies in this consensus. Due to some low quality issues, conflicting opinions, or even lack of evidence. The expert group encourages further exploration of the best population for rhG-CSF, dosage, administration scheme, start and end points, and long-term safety through multicenter clinical observation and clinical data analysis, and continues to improve drug mechanisms, indications, and possible adverse drug reactions and precautions in clinical practice. The expert group will timely supplement and update according to the latest research evidence and information.

**5.3** This consensus strongly recommends that doctors and patients fully communicate about the etiology, indications and adverse reactions of medication, possible pregnancy outcomes, and risks, and inform patients that there is currently no evidence of an association between rhG-CSF and genetic abnormalities, embryonic abnormalities, and ectopic pregnancy. After signing the informed consent for off-label use of drugs and treatment, rhG-CSF can be used for patients with thin endometrium, URSA, URIF, slow hCG rise, and delayed embryo development who have failed routine treatment.

### Abbreviations

hCG, human chorionic gonadotropin; ICSI, Intracytoplasmic sperm injection; IL-10, interleukin-10; IVF-ET, in vitro fertilization-embryo transfer; LIF, leukemia inhibitory factor; PBMC, autologous fresh peripheral blood mononuclear cells; rhG-CSF, recombinant human granulocyte colony-stimulating factor; RIF, recurrent implantation failure; RSA, recurrent spontaneous abortion; TNF-α, tumor necrosis factor-α; URSA, unexplained recurrent spontaneous abortion; VEGF, vascular endothelial growth factor.

## Conflict of interest

All authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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## Authors' contributions

JMC contributed to the consensus concept and design, ZHZ, YZJ were responsible for data acquisition, JMC, ZHZ wre responsible for manuscript drafting. All authors approved the final version of the manuscript.

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