Role of Granulocyte Colony Stimulating Factor on Implantation in Women with Recurrent Implantation Failure in ICSI Cycles.

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ABSTRACT
Recurrent implantation failure (RIF) poses a significant challenge in assisted reproductive technology (ART), defined as the failure to achieve pregnancy following multiple embryo transfers. This review examines the role of Granulocyte Colony-Stimulating Factor (G-CSF) in improving implantation outcomes in women with RIF undergoing ICSI cycles. G-CSF, a cytokine involved in neutrophil regulation, has shown potential in enhancing endometrial receptivity, modulating immune responses, and promoting embryo implantation. Preclinical studies suggest G-CSF influences endometrial thickness, trophoblast invasion, and angiogenesis, while clinical trials report mixed results on its efficacy in improving pregnancy rates. This review explores the physiological mechanisms of G-CSF in reproduction, its therapeutic applications in RIF, and the need for further high-quality evidence to confirm its benefits. Additionally, the review addresses other contributing factors to RIF, including immune dysregulation, genetic abnormalities, anatomical issues, and endocrine imbalances, offering a comprehensive overview of current understanding and treatment strategies for RIF.

Introduction

RIF mentions to the repeated inability of an embryo to implant to a detectable stage, as recognized through pelvic ultrasonography (Simon et al., 2012). According to Coughlan et al. (2014), RIF is the inability of women under 40 for achieving a clinical pregnancy following transferring as a minimum four high-quality embryos over the course of at least three fresh or frozen cycles. Numerous molecular interactions among embryo and endometrium are necessary for effective embryo implantation, which is a complex biological process (Khodry et al., 2024). In this intricate process, endometrial integrins, growth factors, adhesion molecules, extracellular matrix molecules, and ion channels are essential elements (Davidson & Coward, 2016).

G-CSF is part of the colony-stimulating factor family and is synthesized by a variety of cell types, involving endothelial cells, fibroblasts, macrophages, and lymphocytes (Würfel, 2000). G-CSF is also present in reproductive tissues, involved the human ovary and endometrium (Zhao et al., 2016). Preclinical research has demonstrated that luteinized granulosa cells contain G-CSF and its receptor, placental trophoblasts, and oocytes, suggesting its role in reproductive processes (Cai et al., 2015).

G-CSF is believed to play several physiological roles in pregnancy, including promoting embryo cleavage and blastocyst formation (Cai et al., 2015), regulating key endometrial functions essential for implantation—including reshaping blood vessels, influencing the immune system, and promoting cellular attachment (Rahmati et al., 2014)—and influencing follicle development and ovulation (Salmassi et al., 2004). Despite its potential, randomized controlled trials have produced conflicting results regarding G-CSF's effects on rates of endometrial thickness, implantation rates, and rates of clinical pregnancy after in vitro fertilization (Aleyasin et al., 2016).

Research on GM-CSF, a related factor, supports its importance in fetal growth and survival. Studies on animal models have demonstrated that the absence of GM-CSF caused higher pregnancy loss rates and impaired survival of offspring (Savion et al., 2002). GM-CSF-supplemented culture media has been shown to enhance embryo viability by promoting larger inner cell masses and reducing apoptosis, with no negative effects on ploidy rates or chromosomal integrity (Sjöblom et al., 2002). GM-CSF-supplemented media is now commercially obtainable and widely used in assisted reproduction as an adjunctive therapy to improve IVF success rates (Armstrong et al., 2020).

Physiology of Implantation

During implantation, molecular and physiological processes facilitate a two-way conversation among embryo and an open endometrium. Procedure of implantation is multi-staged and intricate:

1. **Fertilization:** The sperm fertilizes the oocyte to form a zygote, that develops into a blastocyst containing an inner cell mass (embryo precursor) and trophoblasts (placenta precursor), migrating to the uterine cavity for implantation (Woodward, 2018).
2. **Endometrial Structure:** The endometrium, the uterine lining, consists of stromal, epithelial, endothelial, and immune cells. It thickens during the proliferative phase under estrogen and becomes receptive to implantation during the secretory phase under progesterone influence (Grund & Grümmer, 2018).
3. **Window of Uterine Receptivity:** Implantation occurs during a specific period, the window during which the endometrium implants expresses pinopodes and molecules like leukemia inhibitory factor (LIF), progesterone, and integrin αVβ3, which promote embryo attachment and invasion (Governini et al., 2021).
4. **Mediators of Implantation:** Growth factors, cytokines, and adhesion molecules, such as CG, LIF, interleukin-6 (IL-6), and mucin-1 (MUC-1), regulate hormonal responses and cell interactions essential for successful implantation (Günther et al., 2023).

Dynamics of Implantation

Throughout the window for implantation, the endometrium expresses a number of genes that facilitate the implantation process. The uterus undergoes substantial tissue remodeling that bears resemblance to a micrometastatic process (Mor, et al. 2017).

The blastocyst attaches to the apical surface of the epithelium upon entering the uterine fundus and subsequently penetrates the luminal epithelium to invade the stroma. Implantation can be categorized into four distinct stages: immunological modulation, adhesion/attachment, apposition, and invasion/penetration (Su and Fazleabas 2015) (Figure 2):

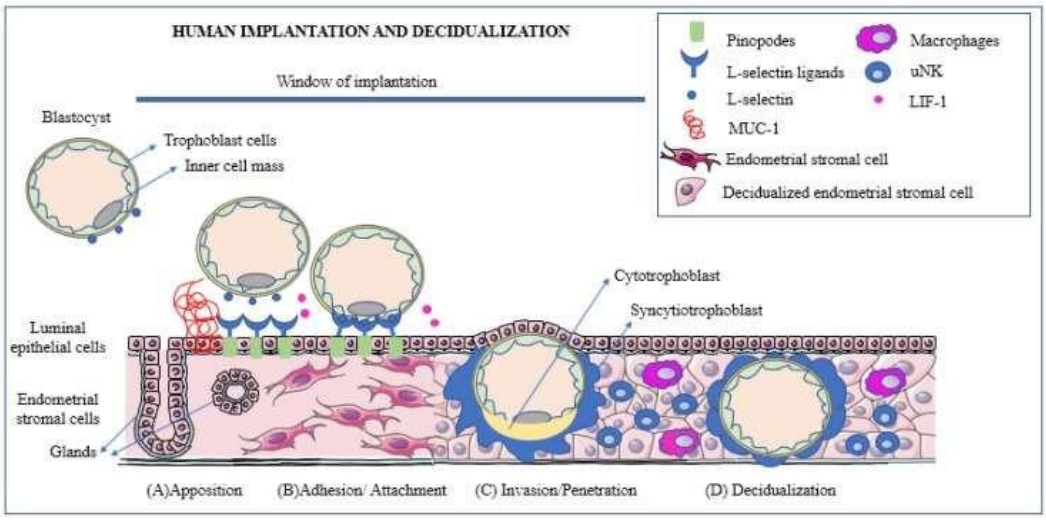

Figure (1): Stages of human implantation (Ochoa-Bernal and Fazleabas 2020).

- **Apposition:** During apposition, the blastocyst loosely interacts with the receptive endometrium, aided by receptor-ligand interactions. Selectins, particularly L-selectin, mediate the blastocyst's rolling and tethering, while the inner cell mass orientation aligns with the endometrium. MUC-1, an anti-adhesion molecule, ensures proper attachment by preventing unwanted attachment before implantation (Ashary et al., 2018).
- **Adhesion/Attachment:** Successful adhesion requires the removal of mucins like MUC-1, which are cleaved at the implantation site. Cytokines, notably LIF, perform an essential function in attracting the blastocyst to the implantation site. Integrins, particularly αVβ3, are essential for blastocyst attachment to the endometrium and proper implantation, with abnormal expression linked to infertility and pregnancy loss (Salama & Alloush, 2020; Joshi et al., 2021).
- **Invasion/Penetration:** Trophoblast cells through the invasion tend to penetrate the endometrial epithelium, forming invadopodia that degrade the basement membrane. The syncytiotrophoblast invades the epithelium, a process called syncytialization. The trophoblast cells proliferate and differentiate, leading to placentation, where primary villi form and eventually develop into secondary and tertiary villi (Ochoa-Bernal & Fazleabas, 2020; Huppertz, 2023).

Decidualization: Decidualization is the transformation of uterine stromal cells into decidual cells, crucial for pregnancy establishment. This process is regulated by progesterone and involves immune tolerance, tissue remodeling, and angiogenesis. Uterine natural killer cells and the Notch signaling pathway also play vital roles in regulating the process, which ensures controlled trophoblast invasion and maternal safety during pregnancy (Ng et al., 2020; Elagab et al., 2022; Orzechowska et al., 2020; Okada et al., 2018).

Recurrent Implantation Failure

RIF refers to repeated failure of embryo implantation, despite multiple attempts. Although no universal definition exists, some suggest RIF as failure for achieving clinical pregnancy following transferring ten or more

embryos, while others recommend basing it on transferring 3 or more embryos, considering improved implantation rates with advances in IVF and blastocyst transfer (Coughlan et al., 2014). Research has highlighted the rarity of RIF following 3 successful euploid embryo transfers in a normal endometrium, indicating that most failures may be linked to embryonic factors rather than endometrial causes (Pirtea et al., 2021).

IVF cycle failures can arise from a combination of factors, including immune system responses, genetic issues, anatomical and hematologic factors, microbiome imbalances, and hormonal influences. These elements affect embryo-endometrial synchrony, making the process of implantation complex and multifactorial (Franasiak et al., 2021).

Factors causing RIF:

Immune factors in RIF

Recent research has focused on the role of endometrial immune cells in predicting IVF outcomes, highlighting the importance of immune interactions at the maternal-fetal interface (Iwes et al., 2023). Dysregulation in the communication among trophoblasts, macrophages, and uNK cells may contribute to implantation failure (Zhang and Wei, 2021).

An endometrial immune profile is proposed to detect immune dysregulation linked to RIF, as immune cells in the endometrium respond to hormonal changes and fetal antigens. Uterine NK cells are important for regulating vascular remodeling and trophoblast invasion, while maternal KIR interactions with fetal HLA-C molecules influence invasion. Women with the KIR AA genotype may face increased risk of pregnancy complications due to insufficient immune activation (Mukherjee et al., 2023; Yang et al., 2020).

Uterine tissue disease like endometriosis, chronic endometritis and adenomyosis, as well as systemic disorders like obesity, PCOS, and autoimmunity, can alter endometrial immune profiles, contributing to implantation failure. Environmental and inflammatory signals can disrupt immune cell function and transcription of genes in the endometrium, leading to tissue dysregulation (Lambert, et al. 2016).

Genetic factors in RIF

Rare genetic disorders include chromosomal aneuploidy and rearrangements are causes of early and recurrent pregnancy loss, but their role in RIF remains less clear (Albaghdadi and Kan, 2021). Maternal age is a key factor, as it increases errors in meiosis, leading to higher aneuploidy rates in embryos, though selective transfer of euploid embryos can improve fertility outcomes. Mosaic embryos, containing both normal and abnormal cells, also show reduced implantation success. Sperm DNA fragmentation was suggested as a reason for recurrent implantation failure, though studies are inconclusive due to testing inconsistencies (Xi et al., 2022).

Maternal thrombophilia like factor V Leiden and MTHFR mutations have been associated with implantation failure in some investigations. Additionally, research suggests that the KIR A inhibitory haplotype may provide protection against RIF. Variations in the ANXA5 gene, such as the M2 haplotype, may also contribute to RIF, though evidence is limited. ERA, which evaluates gene expression in the endometrium, holds potential for personalizing the window of implantation, but further validation is needed (Sato et al., 2019).

Anatomic factors in RIF

Embryo implantation may be hampered by anatomic anomalies such as hydrosalpinges, fibroids, polyps, intrauterine adhesions, and Müllerian malformations (Morin, et al. 2017):

Fibroids:

Fibroids can negatively impact implantation through mechanisms like venous congestion, reduced blood supply, and increased myometrial contractions, with effects varying by location (Ali et al., 2018). Submucosal fibroids significantly reduce pregnancy rates after IVF, while myomectomy can improve outcomes. However, subserosal fibroids and noncavity-distorting intramural fibroids generally do not affect statistics on clinical pregnancies or live births (Barcelos et al., 2021; Yan et al., 2018).

The role of myomectomy for intramural fibroids is debated, with studies showing mixed results on its impact on fertility. Guidelines suggest myomectomy may be unnecessary for asymptomatic, noncavity-distorting intramural fibroids but may be considered in select cases (Metwally et al., 2020; Penzias et al., 2017).

Polyps:

Uterine lesions commonly found are endometrial polyps linked to recurrent implantation failure (RIF), associated with changes in molecular markers such as elevating glycodelin levels while decreasing HOXA10 and IL10 levels. Histologic abnormalities in the endometrium of females with polyps were stated. A randomized controlled trial showed a twofold rise in the likelihood of becoming pregnant following hysteroscopic polypectomy in women undergoing intrauterine insemination, regardless of polyp size (Bashiri et al., 2018).

However, RCTs on polypectomy prior to IVF are non-existent, and retrospective investigations and meta-analyses haven't consistently shown improvements in live birth rates or clinical pregnancy. Diagnostic hysteroscopy alone appears to improve IVF outcomes. To find out if polypectomy can improve implantation, more research is required based on polyp size and location, with office hysteroscopic polypectomy recommended for polyp excision (Ghaffari et al., 2016).

Uterine Septum

The majority of infertile women have a septate uterus, a type of Müllerian abnormality, is linked to decreased clinical pregnancy rates (CPR), increased miscarriage rates (MR), preterm births, and malpresentation at delivery (Mohamed et al., 2019). These complications may be due to a lack of proper blood flow, problems with placentation, decreased steroid sensitivity, and irregular uterine contractions. While observational studies suggest that hysteroscopic septoplasty improves pregnancy results, involving lesser MR and greater CPR, these studies often lack control groups and have inconsistent diagnostic criteria and follow-up (Franasiak et al., 2021).

The only randomized controlled trial (RCT) on septoplasty, Results from the Randomized Uterine Septum Transection Trial did not indicate a statistically significant increase in pregnancies number that continued following the procedure, live birth rates (LBR), or preterm delivery after septum resection. However, concerns about the diagnostic criteria used, such as the removal of septum length restrictions during the study, may have impacted the results. While the ASRM guidelines suggest considering hysteroscopic septoplasty for infertile patients, further RCTs with consistent criteria are needed to provide clear evidence-based treatment recommendations (Rikken et al., 2021; Ludwin et al., 2021).

Intrauterine Adhesions

Asherman syndrome is an example of an intrauterine adhesion that affects 38% of women who have early pregnancy loss and 8% of infertile people. These adhesions can obstruct the cervical canal or fallopian tubes, impair placental growth, and interfere with the implantation and transportation of sperm. According to a study including 2,151 women with Asherman syndrome, 43% of them had infertility (Franasiak et al., 2021). Despite the fact that there aren't any trusted randomized controlled trials (RCTs) on hysteroscopic adhesiolysis, cohort studies support its effectiveness, with pregnancy rates after surgery ranging from 32% to 81%, depending on the severity of the adhesions.

For women with RPL, hysteroscopic adhesiolysis has been correlated with a significant rise in LBR, between eighteen percent and sixty-four percent. A systematic review of thirty-six investigations found a 63% pregnancy probability following adhesiolysis and a 75% live birth rates between cases conceived. Despite favourable outcomes, females with Asherman syndrome keep on at risk for complications such as abnormal placentation, uterine rupture, and preterm birth (Yu et al., 2008; Katz et al., 1996; Deans and Abbott, 2010).

Hydrosalpinx

Hydrosalpinx significantly reduces CPR, implantation rates, and LBR by 50% in females suffering in vitro fertilization, primarily due to the toxic effects of hydrosalpinx fluid, which can impair endometrial receptivity and cause mechanical flushing. Salpingectomy remains the gold standard for managing hydrosalpinx, doubling CPR; however, two randomized controlled trials (RCTs) have shown comparable IVF outcomes with proximal tubal occlusion. Alternatives like hydrosalpinx fluid aspiration and sclerotherapy have been evaluated, with some studies suggesting improved CPR, though the quality of evidence is lower. Aspiration, while offering some benefit, often results in fluid reaccumulation, while sclerotherapy has shown better results in improving CPR and implantation rates (Ali et al., 2022). The ASRM currently suggests proximal tubal occlusion or salpingectomy as a management for females suffering IVF who have hydrosalpinges, though alternatives like aspiration, sclerotherapy, and even neosalpingostomy for select cases may also be considered effective (Volodarsky-Perel et al., 2019; Melo et al., 2020; Cohen et al., 2018).

Hematologic factors in RIF

- **Inherited Thrombophilia:** Inherited thrombophilia, including mutations in factors like FVL, prothrombin gene, and MTHFR, as well as deficiencies in natural anticoagulants involved protein C, protein S, and antithrombin III have been studied for their potential association with RIF. While some investigations have found a higher frequency of these mutations in females with RIF, other larger studies and meta-analyses have not supported this link. The evidence remains inconclusive regarding their role in RIF (Diaz-Nuñez, et al., 2019).
- **Acquired Thrombophilia:** Acquired thrombophilia, particularly antiphospholipid antibodies (aPLs), have been implicated in RPL and other obstetric complications, but their role in RIF is less clear. Some studies suggest an association between aPLs and RIF, while others do not. Due to the conflicting evidence, the

guidelines of the Canadian Fertility and Andrology Society don't support testing for inherited or acquired thrombophilia in females with recurrent implantation failure (Chrisostomos, et al., 2021).

Endocrine factors in RIF:

- **Luteal Phase Defect:** Luteal phase defect, where inadequate corpus luteum function leads to suboptimal progesterone levels, can impair endometrial preparation for implantation. However, robust evidence supporting luteal phase defect as a direct reason for implantation failure remains deficient (Suthaporn, et al., 2021).
- **Supraphysiologic Estrogenic Milieu throughout Ovarian Stimulation:** Controlled ovarian hyperstimulation often results in elevated estrogen levels, which may lead to premature progesterone elevation and asynchronous endometrial development, potentially hindering implantation. Studies have shown disruptions in the endometrial transcriptome and histology in cycles with premature progesterone elevation (Uppangala, et al., 2020).
- **Supraphysiologic Estrogen Concentrations in Frozen Embryo Transfer Cycles:** High estradiol levels during artificial preparation cycles for frozen embryo transfer may affect the luteal transition and implantation, though studies have not consistently demonstrated adverse effects on implantation success (Mackens, et al., 2020).
- **Length and Timing of Progesterone Administration in Frozen Embryo Transfer Cycles:** Progesterone exposure duration in frozen embryo transfer cycles is crucial for endometrial receptivity. However, the prevalence of issues related to progesterone administration remains uncertain (Zhang, et al., 2023).

Granulocyte Colony-Stimulating Factor (G-CSF)

G-CSF is a cytokine primarily involved in stimulating the proliferation and differentiation of neutrophils in the bone marrow. It also plays a role in promoting their release into the bloodstream and enhancing neutrophil functions such as phagocytosis and oxidative processes. First discovered in rats in 1983 and later purified in humans in 1986, granulocyte colony-stimulating factor interacts with its specific receptor found on various cells, including myeloid progenitors, mature neutrophils, platelets, monocytes, and some lymphoid cells, as well as non-hematopoietic cells involved endothelial and trophoblastic cells (Theyab, et al., 2021; Eftekhar, et al., 2018).

Emerging research highlights the significant role of G-CSF in pregnancy success. It is involved in promoting embryo implantation, improving ovarian function, and enhancing endometrial thickening. G-CSF also plays a key role in the pathophysiology of endometriosis, thereby reducing pregnancy loss. Its effects on implantation have led to its consideration as a remedy for implantation failure. Additionally, G-CSF impacts decidual macrophages, ovulation, and granulosa cell functions, in addition to enhancing stimulation of ovary in poor responders, making it a useful marker for IVF outcomes and potentially predictive of oocyte or embryo implantation success (Eftekhar, et al., 2016b; Miralaei, et al., 2019).

G-CSF is also implicated in several reproductive conditions, including luteinized unruptured follicle (LUF) syndrome and recurrent spontaneous abortion. Its therapeutic potential in reducing repeated pregnancy

loss and improving outcomes in women with repeated IVF failure has been recognized. Furthermore, G-CSF has a role in the development of early endometriotic lesions and has immunomodulatory effects that suppress autoimmunity. Serum G-CSF levels remain elevated during pregnancy, with its production originating from fetal chorionic villi and decidual tissues of the mother throughout the initial three months of pregnancy (Lian, et al., 2020).

Function and biology of granulocyte-colony stimulating factor

Numerous cells in the female reproductive system, including fibroblasts, granulosa cells, macrophages, natural killer cells, epithelial apical cells, and trophoblasts, produce G-CSF. On the surface of many different cell types, such as B and T cells, endothelial cells, , and granulosa-lutein cells, the G-CSF receptor (G-CSFR) is widely expressed. In the first trimester, syncytiotrophoblasts, cytotrophoblasts, and extravillous trophoblasts in the placenta have considerable expression of both G-CSF and G-CSFR. Furthermore, invading stromal cells and extravillous trophoblasts within the decidua contain G-CSF and its receptor (Ding, et al., 2021).

Within follicular fluid, G-CSF is predominantly expressed in granulosa cells. Research has shown that G-CSF plays a key role in promoting stem cell migration, differentiation, and mobilization. It also regulates endometrial regeneration through processes such as apoptosis and angiogenesis by binding to granulocyte-colony stimulating factor receptor, which triggers various signaling pathways. Furthermore, G-CSF is vital for metabolism of the placenta, formation of trophoblasts, decidualization of endometrial stromal cells, & ovulation. These findings highlight the significance of granulocyte-colony stimulating factor in both abnormal & normal pregnancies (Kamath, et al., 2017).

Role Of G-CSF in Curing Normal Infertile Females

Rates of implantation remain relatively low despite advancements in assisted reproductive technology. An effective embryo transfer process, a receptive endometrium, and a high-quality embryo are all necessary for successful implantation (Eftekhar et al., 2016a).

In a parallel, randomized, double-blinded, placebo-controlled clinical study by Barad et al., 73 normal in vitro fertilization cases had granulocyte colony-stimulating factor, while sixty-eight others received a placebo. The study showed that G-CSF treatment significantly increased endometrial thickness by 1.36 mm, but it did not improve clinical pregnancy rates, implantation rates, or the overall success of IVF (Barad et al., 2014).

To further evaluate the impact of granulocyte colony-stimulating factor on in vitro fertilization results, Eftekhar et al. (2016a) conducted research with infertile females who had normal endometrial thickness. Within this study, 50 women received three hundred micrograms of transcervical intrauterine G-CSF on the day of oocyte retrieval, while the other 50 women followed the standard IVF protocol. The results revealed that G-CSF treatment didn't enhance chemical pregnancy, rates of clinical pregnancy, continued pregnancy, implantation, or miscarriage in individuals undergoing routine IVF who have normal endometrial thickness.

The mechanism of granulocyte colony-stimulating factor management in reproduction

Previous studies recommend that G-CSF can play an important role in improving reproductive

outcomes, although the exact mechanism remains unclear. It has been shown that G-CSF administration may rise endometrial thickness and elevate the expression of key proteins and molecules such as vimentin, cytokeratin 19, VEGF, PCNA, LIF, and vascular endothelial growth factor receptor-2. These changes promote endometrial proliferation, angiogenesis, and DNA fragmentation, all of which are critical for implantation (Xie et al., 2020).

In experimental porcine models, G-CSF supplementation has been found to upregulate the expression of genes associated with BCL2, PCNA, and POU5F1in blastocyst samples. Furthermore, human endometrial biopsies reveal which granulocyte colony-stimulating factor appears for modulating essential genes involved in embryo adhesion, cell migration, tissue remodeling, and angiogenesis which are essential for successful placentation & embryo implantation. These genes include granulocyte colony-stimulating factor receptor, ITGB3, PLAUR, and TYMP (Cai et al., 2024).

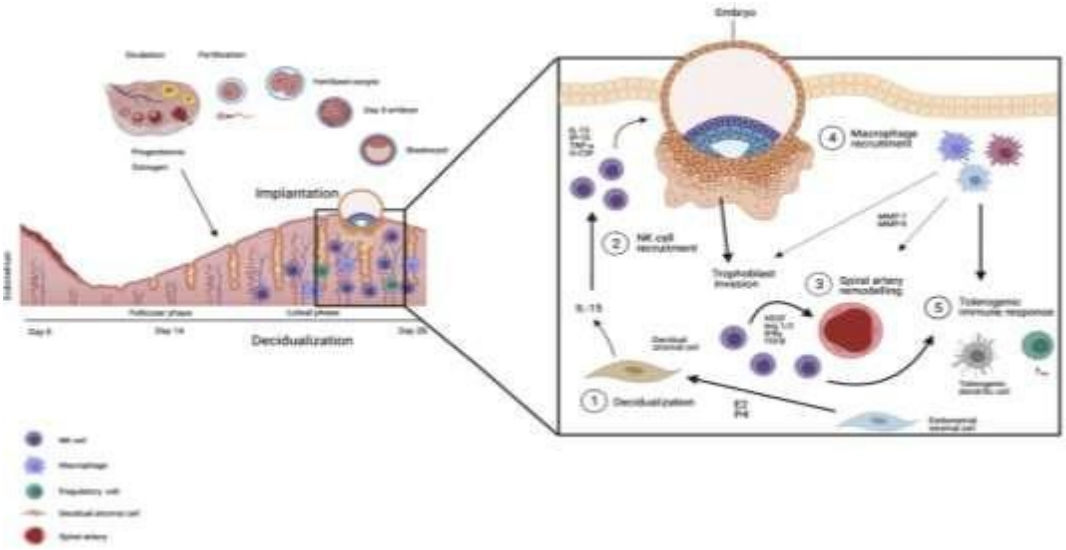

Figure (2): The mechanism of granulocyte colony-stimulating factor management in reproduction (Genest et al., 2022).

Additionally, its effects on the blastocyst & endometrium, studies have shown that G-CSF influences trophoblast function through the activation of key signaling pathways involved Erk1/2, PI3K/Akt, and p38. These pathways enhance MMP-2 activity, VEGF secretion, and β1 integrin expression, while also promoting trophoblast invasion and migration through epithelial-mesenchymal transition. G-CSF further promotes trophoblast viability and proliferation via p38/Erk1/2 and Jak/STAT activation. Additionally, G-CSF-G-CSFR interaction boosts VEGF secretion, aiding placental blood vessel formation, and modulates immune responses by affecting lymphocytes, macrophages, T regulatory cell, and Th2 cells. It increases CD4+ and CD8+ T cell levels, stimulates FOXP3 expression in Tregs, and upregulates granulocyte colony-stimulating factor, vascular endothelial growth factor, and granulocyte colony-stimulating factor receptors on trophoblasts. Nevertheless, further research is needed to fully understand the mechanisms through which G-CSF impacts reproduction (Ding et al., 2021; Wen et al., 2019).

G-CSF in women with RIF

The use of G-CSF in RIF was originally stated Scarpellini et al. (Scarpellini and Sbracia 2012) who discovered that granulocyte colony-stimulating factor treatment greatly increased the pregnancy rate in RIF when compared to the control group. This result was supported by later research. Further research, however, produced negative results. G-CSF can significantly increase the pregnancy rate in patients with recurrent implantation failure, according to several meta-analyse (Jiang, et al. 2020).

The effectiveness of subcutaneous versus intrauterine G-CSF perfusion in individuals with RIF was assessed in retrospective research. The results showed that in RIF patients, systemic G-CSF treatment functioned better than intrauterine perfusion. Additionally, their most current research showed which the combined injection of systemic and intrauterine granulocyte colony-stimulating factor may increase the effect of granulocyte colony-stimulating factor on outcomes of pregnancy. The effects of intrauterine granulocyte colony-stimulating factor on pregnancy outcomes of 157 individuals with normal endometrium and recurrent implantation failure were investigated in a recent randomized controlled experiment. They demonstrated that neither clinical pregnancy rates nor live birth rates were improved by intrauterine G-CSF perfusion (Zeyneloglu, et al. 2020).

Furthermore, RIF patients may achieve comparable pregnancy outcomes to those of healthy women with the administration of intrauterine perfusion of PRP combined with subcutaneous G-CSF injections. Currently, there is a limited amount of high-evidence study about the utilization of G-CSF in cases with RIF, due to the heterogeneity across individuals and the variations in granulocyte colony-stimulating factor dosage, administration methods, and treatment duration reported. Consequently, additional high-quality evidence is required to validate the therapeutic efficacy of granulocyte colony-stimulating factor in cases with RIF (Dieamant, et al. 2019).

A multicenter, randomized, controlled trial by Aleyasin et al. indicates that the administration of a single-dose systemic subcutaneous G-CSF before implantation may markedly enhance implantation and pregnancy rates in infertile females with a history of recurrent IVF failure. (Aleyasin, et al. 2016a).

60 mg of G-CSF was administered daily to 109 cases with RIF in a randomized controlled study in 2012. This regimen was continued for an extra forty days following a positive pregnancy test. There was a highly significant difference between the G-CSF group's clinical pregnancy rate per embryo transfer of 43.1% and the placebo group's (saline injection) 21.6%. There were no negative side effects noted. Furthermore, there was a notable difference in probabilities of pregnancy between day two and day five embryo transfers (Scarpellini and Sbracia 2012).

The efficacy of transvaginal G-CSF perfusion in situations of repeated implantation failure was evaluated by Eftekhar et al. (2016). Following intrauterine infusion of G-CSF, the intervention group experienced a statistically significant improvement in pregnancy outcomes.

G-CSF for women with infertility

A randomized controlled study by Barad et al. found that intrauterine infusion of G-CSF didn't enhance implantation or clinical pregnancy rates in fresh embryo transfer cycles among cases undergoing standard IVF treatment. The conclusion that intrauterine G-CSF infusion did not improve pregnancy outcomes in healthy IVF

women with sufficient endometrial thickness was supported by a different RCT. Consequently, it is unclear how G-CSF injections affect the clinical pregnancy and loss rates in unselected infertile women undergoing IVF. G-CSF is an immunogenic treatment that mainly stimulates neutrophil differentiation and proliferation while also influencing decidual macrophages (Kamath, et al. 2020).

These patients have never had IVF cancellation or failure due to a thin endometrium. Therefore, the results of these patients' pregnancies may not be affected by intrauterine G-CSF injections. However, in individuals who have experienced two or more IVF failures, G-CSF medication may increase the clinical pregnancy rate. By enhancing the fertilization capacity of oocytes through a local paracrine effect, G-CSF supplementation significantly improves pregnancy outcomes in women who respond poorly to IVF. G-CSF may benefit patients who have experienced two or more IVF failures when taken together, however its effectiveness in treating healthy IVF patients is not very strong (Huang, et al. 2022).

Granulocyte colony-stimulating factor for other pregnancy-correlated illnesses

Granulocyte colony-stimulating factor may be a beneficial treatment for luteinized unruptured follicle syndromes (LUFs), regarding previous research by Takeo Shibata et al. (Shibata et al. 2016). Recombinant G-CSF given before human chorionic gonadotropin may be able to prevent ninety percent of luteinized unruptured follicle syndromes in clomiphene citrate cycles. Granulocyte colony-stimulating factor treatment throughout pregnancy may decreased the incidence of preterm birth and preeclampsia, according to research. The addition of granulocyte colony-stimulating factor to culture medium significantly increases clinical pregnancy and implantation rate, improves development and post-transfer survival, and decreases pregnancy loss as compared to standard media. Furthermore, more research is needed to determine how G-CSF functions in other pregnancy-related conditions.

References

[1] Albaghdadi AJ, Kan FW. (2021). Therapeutic potentials of low-dose tacrolimus for aberrant endometrial features in polycystic ovary syndrome. International Journal of Molecular Sciences, 22(6): 1-17.

[2] Aleyasin A, Abediasl Z, Nazari A, Sheikh M. (2016). Granulocyte colony-stimulating factor in repeated IVF failure, a randomized trial. Reproduction, 151(6): 637-642.

[3] Ali AENA, Taha SA, El-Ghany A, Mohammed F, El-Sayed AEHM. (2018). Three-dimensional transvaginal U/S measurements of uterine junctional zone thickness and its relation to implantation success rate in ICSI cycles. SVU-International Journal of Medical Sciences, 1(1): 17-22.

[4] Ali AENAEG, Abdellah AH, Mohammed AH, Ahmed HH. (2022). Soft versus conventional protocol in ovarian stimulation in intracytoplasmic sperm injection cycles for poor responders: A randomized clinical trial. SVU-International Journal of Medical Sciences, 5(1): 188-196.

[5] Armstrong S, MacKenzie J, Woodward B, Pacey A, Farquhar C. (2020). GM-CSF (granulocyte macrophage colony-stimulating factor) supplementation in culture media for women undergoing assisted reproduction. Cochrane Database of Systematic Reviews, 1(7): 1-57

[6] Ashary N, Tiwari A, Modi D. (2018). Embryo implantation: war in times of love. Endocrinology, 159(2): 1188-1198.

[7] Barad DH, Yu Y, Kushnir VA, Shohat-Tal A, Lazzaroni E, Lee HJ, et al. (2014). A randomized clinical trial of endometrial perfusion with granulocyte colony-stimulating factor in in vitro fertilization cycles: impact on endometrial thickness and clinical pregnancy rates. Fertility and Sterility, 101(3): 710-715.

[8] Barcelos MFR, da Rocha Filho AM, Goulart AEC, Souza ALM, Costa DT, Barros GG, et al. (2021). Recurrent implantation failure: the role of anatomical causes. IntechOpen, 1-244.

[9] Bashiri A, Halper KI, Orvieto R. (2018). Recurrent implantation failure-update overview on etiology, diagnosis, treatment and future directions. Reproductive Biology and Endocrinology, 16(1): 121-139.

[10] Cai L, Jeon Y, Yoon JD, Hwang SU, Kim E, Park Km, et al. (2015). The effects of human recombinant granulocyte-colony stimulating factor treatment during in vitro maturation of porcine oocyte on subsequent embryonic development. Theriogenology, 84(7): 1075-1087.

[11] Cai L, Jeong YW, Hwang WS, Hyun SH. (2024). Optimization of human recombinant granulocyte-colony stimulating factor supplementation during in vitro production of porcine embryos to improve the efficiency of resource utilization of poor-quality cumulus-oocyte complexes. Theriogenology, 216(1): 93-102.

[12] Chrisostomos S, Maria P, Sotiris S, Georgios T. (2021). Recurrent pregnancy loss resulting in IVF (In Vitro Fertilization) series. Pathophysiologic mapping. A systematic review. Advances in Hematology and Oncology Research, 4(1): 66-70.

[13] Cohen A, Almog B, Tulandi T. (2018). Hydrosalpinx sclerotherapy before in vitro fertilization: systematic review and meta-analysis. Journal of Minimally Invasive Gynecology, 25(4): 600-607.

[14] Coughlan C, Ledger W, Wang Q, Liu F, Demirol A, Gurgan T, et al. (2014). Recurrent implantation failure: definition and management. Reproductive BioMedicine Online, 28(1): 14-38.

[15] Davidson LM, Coward KJ. (2016). Molecular mechanisms of membrane interaction at implantation. Birth Defects Research Part C: Embryo Today: Reviews, 108(1): 19-32.

[16] Deans R, Abbott J. (2010). Review of intrauterine adhesions. Journal of Minimally Invasive Gynecology, 17(5): 555-569.

[17] Diaz-Nuñez M, Rabanal A, Expósito A, Ferrando M, Quintana F, Soria JM, et al. (2019). Recurrent miscarriage and implantation failure of unknown cause studied by a panel of thrombophilia conditions: increased frequency of FXIII Val34Leu polymorphism. Journal of Reproduction & Infertility, 20(2): 76-82.

[18] Dieamant F, Vagnini LD, Petersen CG, Mauri AL, Renzi A, Petersen B, et al. (2019). New therapeutic protocol for improvement of endometrial receptivity (PRIMER) for patients with recurrent implantation failure (RIF)–A pilot study. JBRA Assisted Reproduction, 23(3): 250-254.

[19] Ding J, Yang C, Zhang Y, Wang J, Zhang S, Guo D, et al. (2021). M2 macrophage-derived G-CSF promotes trophoblasts EMT, invasion and migration via activating PI3K/Akt/Erk1/2 pathway to mediate normal pregnancy. Journal of Cellular and Molecular Medicine, 25(4): 2136-2147.

[20] Eftekhar M, Hosseinisadat R, Baradaran R, Naghshineh E. (2016). Effect of granulocyte colony stimulating factor (G-CSF) on IVF outcomes in infertile women: an RCT. International Journal of Reproductive BioMedicine, 14(5): 341-346.

[21] Eftekhar M, Miraj S, Mojtahedi MF, Neghab N. (2016). Efficacy of intrauterine infusion of granulocyte colony stimulating factor on patients with history of implantation failure: a randomized control trial. International Journal of Reproductive BioMedicine, 14(11): 687-690.

[22] Eftekhar M, Naghshineh E, Khani P. (2018). Role of granulocyte colony-stimulating factor in human reproduction. J Res Med Sci, 23(1): 7-13.

[23] Elagab EA, Alshahrani M, Elbadawi AA, Aedh AI, Osman AM, Osman HM. (2022). Decidual Natural Killer Cells Are Essential for a Successful Pregnancy. Adv Reprod Sci, 10(3): 73-90.

[24] Franasiak JM, Alecsandru D, Forman EJ, Gemmell LC, Goldberg JM, Llarena N, et al. (2021). A review of the pathophysiology of recurrent implantation failure. Fertil Steril, 116(6): 1436-1448.

[25] Genest G, Almasri W, Banjar S, Beauchamp C, Buckett W, Dzineku F, et al. (2022). Immunotherapy for recurrent pregnancy loss: a reappraisal. F&S Rev, 3(1): 24-41.

[26] Ghaffari F, Arabipoor A, Lankarani NB, Hosseini F, Bahmanabadi A. (2016). Hysteroscopic polypectomy without cycle cancellation in IVF/ICSI cycles: a cross-sectional study. Eur J Obstet Gynecol Reprod Biol, 205(1): 37-42.

[27] Governini L, Luongo FP, Haxhiu A, Piomboni P, Luddi A. (2021). Main actors behind the endometrial receptivity and successful implantation. Tissue Cell, 73(1): 1-9.

[28] Grund S, Grümmer R. (2018). Direct cell–cell interactions in the endometrium and in endometrial pathophysiology. Int J Mol Sci, 19(8): 1-22.

[29] Günther V, Allahqoli L, Deenadayal-Mettler A, Maass N, Mettler L, Gitas G, et al. (2023). Molecular Determinants of Uterine Receptivity: Comparison of Successful Implantation, Recurrent Miscarriage, and Recurrent Implantation Failure. Int J Mol Sci, 24(24): 1-18.

[30] Huang P, Yao C, Wei L, Lin Z. (2022). The intrauterine perfusion of granulocyte-colony stimulating factor (G-CSF) before frozen-thawed embryo transfer in patients with two or more implantation failures. Hum Fertil, 25(2): 301-305.

[31] Huppertz B. (2023). Placental Development with Histological Aspects. In The Placenta: Basics and Clinical Significance. Springer, 1-27.

[32] Iwes MS, ELZarka MA, Fayed HM, Abdellah AH, Ahmed MA. (2023). Impact of the follicular fluid Coenzyme Q10 level in women undergoing intracytoplasmic sperm injection (ICSI) on the pregnancy rate. SVU-International Journal of Medical Sciences, 6(2): 279-290.

[33] Jiang Y, Zhao Q, Zhang Y, Zhou L, Lin J, Chen Y, et al. (2020). Treatment of G-CSF in unexplained, repeated implantation failure: A systematic review and meta-analysis. J Gynecol Obstet Hum Reprod, 49(10): 1-8.

[34] Joshi NR, Kohan-Ghadr HR, Roqueiro DS, Yoo JY, Fru K, Hestermann E, et al. (2021). Genetic and epigenetic changes in the eutopic endometrium of women with endometriosis: association with decreased endometrial αvβ3 integrin expression. Mol Hum Reprod, 27(6): 1-14.

[35] Kamath MS, Chittawar PB, Kirubakaran R, Mascarenhas M. (2017). Use of granulocyte-colony stimulating factor in assisted reproductive technology: A systematic review and meta-analysis. Eur J Obstet Gynecol Reprod Biol, 214(1): 16-24.

[36] Kamath MS, Kirubakaran R, Sunkara SK. (2020). Granulocyte-colony stimulating factor administration for subfertile women undergoing assisted reproduction. Cochrane Database Syst Rev, 1(1):1-63.

[37] Katz Z, Ben-Arie A, Lurie S, Manor M, Insler V. (1996). Reproductive outcome following hysteroscopic adhesiolysis in Asherman's syndrome. Int J Fertil Menopausal Stud, 41(5): 462-465.

[38] Khodry MM, Elsaid AA, Ali AENAEG, Al-hasan A, Al-A'abdeen MZ, Fahmy RM, et al. (2024). Serum anti-endometrial antibodies as a biomarker for implantation rate in patients with endometriosis who had recurrent implantation failure and prepared for Intracytoplasmic Sperm Injection. SVU-International Journal of Medical Sciences, 7(1): 38-48.

[39] Lambert M, Hocke C, Jimenez C, Frantz S, Papaxanthos A, Creux H, et al. (2016). Repeated in vitro fertilization failure: Abnormalities identified in the diagnostic assessment. Gynecol Obstet Fertil, 44(10): 565-571.

[40] Lian R, Wang X, Lin R, Zeng H, Zeng Y, Liu S, et al. (2020). Evaluation of granulocyte colony-stimulating factor on the treatment of thin endometrium during frozen-thawed embryo transfer cycles: a retrospective cohort study. Gynecol Endocrinol, 36(4): 370-374.

[41] Ludwin A, Trolice MP, Bhagavath B, Lindheim SR. (2021). Resection of a uterine septum in a normal uterus: Whoa Nellie. Fertil Steril, 115(5): 1140-1142.

[42] Mackens S, Santos-Ribeiro S, Orinx E, De Munck N, Racca A, Roelens C, et al. (2020). Impact of serum estradiol levels prior to progesterone administration in artificially prepared frozen embryo transfer cycles. Front Endocrinol, 11(1): 1-8.

[43] Melo P, Georgiou EX, Johnson N, van Voorst SF, Strandell A, Mol BWJ, et al. (2020). Surgical treatment for tubal disease in women due to undergo in vitro fertilisation. Cochrane Database Syst Rev, 1(10): 1-81.

[44] Metwally M, Raybould G, Cheong YC, Horne AW. (2020). Surgical treatment of fibroids for subfertility. Cochrane Database Syst Rev, 1(1): 1-44.

[45] Miralaei S, Ashrafi M, Arabipoor A, Zolfaghari Z, Taghvaei S. (2019). The incidence rate of unresponsive thin endometrium in frozen embryo transfer cycles: A case-series of therapy with granulocyte colony stimulating factor. Int J Reprod BioMed, 17(12): 923-928.

[46] Mohamed AM, Mohammad MA, Khodry MM, Abdellah AH. (2019). Day 3 versus day 5 embryo freezing: which is better? A comparative study. SVU-International Journal of Medical Sciences, 2(2): 10-16.

[47] Mor G, Aldo P, Alvero AB. (2017). The unique immunological and microbial aspects of pregnancy. Nat Rev Immunol, 17(8): 469-482.

[48] Morin SJ, Treff NR, Tao X, Scott RT III, Franasiak JM, Juneau CR, et al. (2017). Combination of uterine natural killer cell immunoglobulin receptor haplotype and trophoblastic HLA-C ligand influences the risk of pregnancy loss: a retrospective cohort analysis of direct embryo genotyping data from euploid transfers. Fertil Steril, 107(3): 677-683.e2.

[49] Mukherjee N, Sharma R, Modi D. (2023). Immune alterations in recurrent implantation failure. Am J

[50] Ng SW, Norwitz GA, Pavlicev M, Tilburgs T, Simón C, Norwitz ER, et al. (2020). Endometrial decidualization: the primary driver of pregnancy health. Int J Mol Sci, 21(11): 1-20.

[51] Ochoa-Bernal MA, Fazleabas AT. (2020). Physiologic events of embryo implantation and decidualization in human and non-human primates. Int J Mol Sci, 21(6): 1-21.

[52] Okada H, Tsuzuki T, Murata H. (2018). Decidualization of the human endometrium. Reprod Med Biol, 17(3): 220-227.

[53] Orzechowska M, Anusewicz D, Bednarek AK. (2020). Functional gene expression differentiation of the Notch signaling pathway in female reproductive tract tissues—A comprehensive review with analysis. Front Cell Dev Biol, 8(1): 1-34.

[54] Penzias A, Bendikson K, Butts S, Coutifaris C, Falcone T, Fossum G, et al. (2017). Removal of myomas in asymptomatic patients to improve fertility and/or reduce miscarriage rate: a guideline. Fertil Steril, 108(3): 416-425.

[55] Pirtea P, Cicinelli E, De Nola R, de Ziegler D, Ayoubi JM. (2021). Endometrial causes of recurrent pregnancy losses: endometriosis, adenomyosis, and chronic endometritis. Fertil Steril, 115(3): 546-560.

[56] Rahmati M, Petitbarat M, Dubanchet S, Bensussan A, Chaouat G, Ledee N, et al. (2014). Granulocyte-colony stimulating factor related pathways tested on an endometrial ex-vivo model. PLoS One, 9(10): 1-6.

Reprod Immunol, 89(2): 1-8.

[57] Rikken J, Kowalik C, Emanuel M, Bongers M, Spinder T, Jansen F, et al. (2021). Septum resection versus expectant management in women with a septate uterus: an international multicentre open-label randomized controlled trial. Hum Reprod, 36(5): 1260-1267.

[58] Salama KM, Alloush MK. (2020). Are the cytokines TNF alpha and IL 1Beta early predictors of embryo implantation? Cross-sectional study. J Reprod Immunol, 137(1): 1-18.

[59] Salmassi A, Schmutzler AG, Huang L, Hedderich J, Jonat W, Mettler L, et al. (2004). Detection of granulocyte colony-stimulating factor and its receptor in human follicular luteinized granulosa cells. Fertil Steril, 81(1): 786-791.

[60] Sato T, Sugiura-Ogasawara M, Ozawa F, Yamamoto T, Kato T, Kurahashi H, et al. (2019). Preimplantation genetic testing for aneuploidy: a comparison of live birth rates in patients with recurrent pregnancy loss due to embryonic aneuploidy or recurrent implantation failure. Hum Reprod, 34(12): 2340-2348.

[61] Savion S, Zeldich E, Orenstein H, Shepshelovich J, Torchinsky A, Carp H, et al. (2002). Cytokine expression in the uterus of mice with pregnancy loss: effect of maternal immunopotentiation with GM-CSF. Reprod Camb Engl, 123(3): 399-409.

[62] Scarpellini F, Sbracia M. (2012). G-CSF treatment improves IVF outcome in women with recurrent implantation failure in IVF. J Reprod Immunol, 1(94): 103-110.

[63] Shibata T, Makinoda S, Waseda T, Tomizawa H, Fujii R, Utsunomiya T, et al. (2016). Granulocyte colony-stimulating factor as a potential inducer of ovulation in infertile women with luteinized unruptured follicle syndrome. Transl Res, 171: 63-70.

[64] Simon A, Laufer N. (2012). Repeated implantation failure: clinical approach. Fertil Steril, 97(5): 1039-

1043.

[65] Sjöblom C, Wikland M, Robertson SA. (2002). Granulocyte-macrophage colony-stimulating factor (GM-CSF) acts independently of the beta common subunit of the GM-CSF receptor to prevent inner cell mass apoptosis in human embryos. Biol Reprod, 67(6): 1817-1823.

[66] Su RW, Fazleabas AT. (2015). Implantation and establishment of pregnancy in human and nonhuman primates. Regul Implantat Establish Preg Mammals, 2016(1): 189-213.

Suboptimal mid-luteal progesterone concentrations are associated with aberrant endometrial gene expression, potentially resulting in implantation failure. Reprod Biomed Online, 42(3): 595-608.

[67] Suthaporn S, Jayaprakasan K, Thornton J, Walker K, Medrano JH, Castellanos M, et al. (2021).

[68] Theyab A, Algahtani M, Alsharif KF, Hawsawi YM, Alghamdi A, Alghamdi A, et al. (2021). New insight into the mechanism of granulocyte colony-stimulating factor (G-CSF) that induces the mobilization of neutrophils. Hematology, 26(1): 628-636.

[69] Uppangala S, Fernandes G, Salian SR, Kumar P, Talevi R, Kalthur G, et al. (2020). Reduced ovarian response to controlled ovarian stimulation is associated with increased oxidative stress in the follicular environment. Reprod Biol, 20(3): 402-407.

[70] Volodarsky-Perel A, Buckett W, Tulandi T. (2019). Treatment of hydrosalpinx in relation to IVF outcome: a systematic review and meta-analysis. Reproductive Biomedicine Online, 39(3): 413-432.

[71] Wen Q, Kong Y, Zhao HY, Zhang YY, Han TT, Wang Y, et al. (2019). G-CSF-induced macrophage polarization and mobilization may prevent acute graft-versus-host disease after allogeneic hematopoietic stem cell transplantation. Bone Marrow Transplantation, 54(9): 1419-1433.

[72] Woodward B. (2018). In vitro fertilization and intracytoplasmic sperm injection. Clinical Reproductive Science, 2(1): 1-11.

[73] Würfel WJ. (2000). Approaches to a better implantation. Epub, 17(8): 473-480.

[74] Xi H, Qiu L, Yao Y, Luo L, Sui L, Fu Y, et al. (2022). Noninvasive chromosome screening for evaluating the clinical outcomes of patients with recurrent pregnancy loss or repeated implantation failure. Frontiers in Endocrinology, 13(1): 1-9.

[75] Xie Y, Tian Z, Qi Q, Li Z, Bi Y, Qin A, Yang Y. (2020). The therapeutic effects and underlying mechanisms of the intrauterine perfusion of granulocyte colony-stimulating factor on a thin-endometrium rat model. Life Sciences, 260(1): 1-8.

[76] Yan L, Yu Q, Zhang Yn, Guo Z, Li Z, Niu J, Ma J. (2018). Effect of type 3 intramural fibroids on in vitro fertilization–intracytoplasmic sperm injection outcomes: a retrospective cohort study. Fertility and Sterility, 109(5): 817-822.e12.

[77] Yang X, Yang Y, Yuan Y, Liu L, Meng T. (2020). The roles of uterine natural killer (NK) cells and KIR/HLA-C combination in the development of preeclampsia: a systematic review. BioMed Research International, 1(1): 1-10.

[78] Yu D, Wong YM, Cheong Y, Xia E, Li TC. (2008). Asherman syndrome—one century later. Fertility and Sterility, 89(4): 759-779.

[79] Zeyneloglu HB, Tohma YA, Onalan G, Moran U. (2020). Granulocyte colony-stimulating factor for intracytoplasmic sperm injection patients with repeated implantation failure: which route is best? Journal of Obstetrics and Gynaecology, 40(4): 526-530.

[80] Zhang J, Du M, Wang Z, Wu S, Guan Y, Sun L, et al. (2023). The duration of estrogen treatment before progesterone application does not affect neonatal and perinatal outcomes in frozen embryo transfer cycles. Frontiers in Endocrinology, 14(1): 1-8.

[81] Zhang X, Wei H. (2021). Role of decidual natural killer cells in human pregnancy and related pregnancy complications. Frontiers in Immunology, 12(1): 1-14.

[82] Zhao J, Xu B, Xie S, Zhang Q, Li Y. (2016). Whether G-CSF administration has beneficial effect on the outcome after assisted reproductive technology? A systematic review and meta-analysis. Reproductive Biology and Endocrinology, 14(1): 1-9.