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Impact of Intrauterine Infusion vs. Subcutaneous G-CSF Injection on Endometrial Immunomodulation and Angiogenesis in Infertile Women Undergoing IUI

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Abstract: Infertility remains a significant global health concern, with endometrial receptivity recognized as a critical factor influencing successful implantation. Granulocyte-Colony Stimulating Factor (G-CSF), a hematopoietic cytokine, has recently emerged as a potential therapeutic agent for enhancing endometrial function and modulating immune responses during assisted reproductive procedures. This study aims to investigate the effect of intrauterine infusion versus subcutaneous injection of G-CSF on immunological and angiogenic markers related to endometrial receptivity in infertile women undergoing intrauterine insemination (IUI). A total of 75 infertile females were enlisted in this prospective interventional comparative trial and were equally divided into three groups: the intrauterine infusion group, the subcutaneous injection group, and the control group (no G-CSF administration). Baseline demographic and hormonal characteristics, as well as ultrasound and immunological parameters, were recorded before and after G-CSF administration. Statistical analysis showed no significant differences in demographic and baseline clinical, hormonal, and biochemical characteristics across the groups. Post-intervention, both G-CSF groups demonstrated significant improvements in endometrial vascularity indices (pulsatility index, resistance index, and V1/V2 ratio) compared to the control group, despite the endometrial thickness not differing significantly. Serum levels of TNF-α significantly decreased, while VEGF levels increased significantly post-G-CSF in all groups; IL-10 levels increased but reached significance only in the subcutaneous and control groups. These outcomes indicate that G-CSF may enhance endometrial receptivity via improved vascular and immunological parameters, although the effect remains inconclusive and requires further validation.

Keywords: Endometrial Immunomodulation; Granulocyte Colony-Stimulating Factor; Intrauterine Insemination; Interleukin-10; Tumor Necrosis Factor-Alpha; Vascular Endothelial Growth Factor

1. Introduction

Infertility impacts a considerable percentage of reproductive-age couples globally with multifactorial etiologies, including female, male, combined, or unexplained causes. Female infertility is commonly classified into three major categories: abnormal ovulation, transport issues, and implantation failure. It can be profoundly distressing, leading to significant social, physical, and psychological consequences. Couples facing such challenges often turn to reproductive medicine to address these troubles [1–3]. Comparing assisted reproductive techniques, intrauterine insemination (IUI) is repeatedly considered a first-line treatment due to its relative affordability, minimal invasiveness, and patient-friendly nature [4]. During the procedure, fresh or frozen sperm are introduced into the female

reproductive tract after separating the seminal plasma, using methods other than sexual intercourse. IUI can be performed with or without ovarian stimulation and is particularly indicated for male infertility. Reported pregnancy rates following IUI range between 8.5% and 21.4%, depending on various clinical factors [5,6]. Among female factors, impaired endometrial receptivity is recognized as a critical contributor [7,8]. Moreover, the endometrium is the site of life's creation, and a responsive endometrium exists at the interface of the menstrual phase and pregnancy [9,10]. The embryo's implantation, a distinctive biological process, is the most crucial stage of reproduction [11]. Optimal implantation demands a sensitive endometrium, a functioning and healthy embryo at the blastocyst phase of growth, and a synchronous conversation between the embryo and maternal tissues [12,13]. The membrane of the endometrium is receptive to blastocyst gestation within a limited time and space gap known as the implantation window. This time frame starts between 6 and 10 days after the LH spike and lasts approximately 48 hours [14–16].

A functional and receptive endometrium relies on various factors. Central to this is the synchrony among growth factors, cytokines, and chemokines in the endometrium [17,18]. Cytokines comprise a wide collection of signaling substances that are categorized into various types, including interleukins, chemokines, colony-stimulating factors (CSF), interferons, and a few others [19–22]. Those molecules regulate immunological responses vital for immune system homeostasis, as dysregulated cytokine signaling can lead to undesirable consequences, including inflammatory diseases [23–25]. Type 1 cytokines, notably IL-17, IL-1β, TNF-α, and INF-γ, induce inflammation [26–29], whereas type 2 cytokines, involving IL-10, IL-4, and IL-1ra, modulate this response [30–32]. A physiological equilibrium between these two cytokine types is crucial for successful implantation [33,34].

On the other hand, vascular endothelial growth factor (VEGF) is a signaling substance that stimulates the formation of new blood vessels, both during development and in pathological conditions [35–37]. VEGF works by interacting with particular receptors on endothelial cells, initiating a series of processes that culminate in cell proliferation, emigration, and survival [38–40]. Endometrial vascularization and angiogenic signaling pathways, particularly those involving VEGF, are crucial in establishing a receptive endometrial environment [41]. VEGF is essential for effective embryo implantation throughout assisted reproduction, as it stimulates embryo growth, enhances endometrial receptivity, and facilitates embryo-endometrial interactions [42–44]. Recurrent miscarriage is the primary clinical phenotype linked to VEGF dysregulation [45,46].

Granulocyte-Colony Stimulating Factor (G-CSF) is a complex polypeptide glycoprotein produced by various cell types, comprising bone marrow cells, fibroblasts, endometrial cells, and natural killer cells. Specifically, endometrial cells generate G-CSF during the luteal stage, which provokes vascular reconstruction and decidualization [47]. During pregnancy, G-CSF has been implicated in several physiological roles, including enhancing embryo cleavage and blastocyst formation, regulating endometrial expressions essential for implantation processes involving endometrial vessel remodeling, local immune modulation, and cellular adhesion pathways, as well as promoting follicle development and ovulation [48]. The administration of G-CSF in assisted reproduction is typically done via either subcutaneous injection or intrauterine infusion, each of which may have distinct biochemical and clinical implications. While subcutaneous injection of G-CSF is a well-established route, intrauterine infusion, which directly targets the uterine environment, may provide more localized and potentially more effective results. However, comparative studies evaluating the clinical and biochemical impacts of these two administration methods are limited [49]. Despite the growing application of G-CSF in reproductive medicine, the precise biological mechanisms through which it influences the endometrial milieu remain an area of active investigation. Several clinical trials and animal studies have attempted to elucidate the downstream pathways affected by G-CSF in the reproductive tract, yet many findings remain inconclusive or context-dependent [50,51]. Furthermore, patient variability, including differences in age, hormonal profiles, baseline endometrial thickness, and underlying infertility etiologies, may influence the *in vivo* action of G-CSF [52]. This variability underscores the need for well-structured comparative investigations that consider these clinical variables to understand better the potential role of G-CSF in personalized infertility management. Importantly, immunological and angiogenic markers in the endometrium are key mediators of successful implantation and pregnancy outcomes; thus, analyzing how these parameters are affected by different routes of G-CSF administration may provide meaningful insights into optimizing its clinical use [53].

It has been proposed that G-CSF administration may enhance clinical outcomes following ART treatment; however, it remains unclear which specific infertility problems or routes of administration render G-CSF management beneficial [54]. This work attempted to compare the effects of intrauterine versus subcutaneous G-CSF use on en-

dometrial immunological and angiogenic markers in infertile women who underwent IUI. This work attempted to compare the effects of intrauterine versus subcutaneous G-CSF use on endometrial immunological and angiogenic markers in infertile women who underwent IUI.

2. Patients and Methods

A prospective, interventional, comparative trial was conducted at the Infertility Diagnosis Clinic of the High Institute for Infertility Diagnosis and Assisted Reproductive Technology at Al-Nahrain University from September 1, 2024, to May 1, 2025. The institutional review board ethically approved the study, and informed consent was obtained from all participants prior to their enrollment. A clinical trial database is not applicable. A total of 90 women aged between 18 and 40 years were initially assessed to determine their eligibility for IUI. Each participant underwent a thorough clinical, hormonal, and ultrasonographic evaluation to assess fitness for inclusion. Inclusion criteria included females within the reproductive age group of 18-40 years, diagnosed with either primary or secondary infertility, and confirmed to have a normal ovarian reserve by an Anti-Mullerian Hormone (AMH) level greater than 1.1 ng/mL. Participants were also demonstrated to have either unilateral or bilateral tubal patency, confirmed through diagnostic procedures such as laparoscopy, saline infusion sonography (SIS), or hysterosalpingography (HSG). Additionally, all candidates were required to plan for IUI following a standard ovulation induction protocol. Exclusion criteria included women who had any known or established contraindications to either granulocyte colony-stimulating factor (G-CSF) or IUI procedures; this also encompassed participants with any notable uterine abnormalities, notably submucosal fibroids, polyps, adenomyosis, severe endometriosis, congenital uterine malformation, and cervical stenosis, all of which are known to impact implantation and conception outcomes negatively. These exclusions ensured uniformity and minimized potential confounding variables. After applying the inclusion and exclusion criteria, 15 participants were excluded due to either ineligibility based on medical assessment, personal refusal to participate in the trial, or being lost to follow-up during the preliminary evaluation process. The remaining 75 participants who experienced infertility for a duration exceeding one year were included in the study and subsequently randomized into three equally sized groups (n = 25 each) using a computer-generated randomization table.

- **Group 1:** Received intrauterine infusion of G-CSF at a dose of 300 µg, administered precisely on the day of human chorionic gonadotropin (hCG) trigger.
- **Group 2:** Received subcutaneous injection of G-CSF at the same dose (300 µg) also on the HCG trigger day.
- **Group 3 (control group):** Did not receive any form of G-CSF administration, serving as the untreated comparison group.

2.1. Justification for the Dose (300 µg G-CSF)

The choice of 300 µg of G-CSF was based on prior clinical studies that demonstrated its safety and biological efficacy in improving endometrial receptivity and implantation potential in infertile women undergoing assisted reproductive technologies. Specifically, this dosage corresponds to the standard therapeutic dose of filgrastim (a recombinant G-CSF) commonly used in reproductive medicine, particularly in studies targeting thin endometrium or implantation failure. This dose strikes a balance between safety and efficacy, minimizing the risk of systemic side effects while being sufficient to exert local immunomodulatory and angiogenic effects [55,56].

2.2. Justification for Timing (on the Day of HCG Trigger):

G-CSF was administered on the day of the HCG trigger because this timing aligns with the late follicular phase, just before ovulation and luteinization, when endometrial receptivity is actively developing. Delivering G-CSF at this point aims to enhance local immune tolerance and angiogenesis during the critical pre-implantation window, allow sufficient time for the G-CSF-mediated modulation of the endometrial environment before embryo arrival and implantation, and synchronize the immune and vascular effects of G-CSF with the luteinizing hormone surge induced by hCG, potentially supporting more coordinated endometrial maturation [57,58]. Therefore, the selection of a 300-µg dose and its administration on the HCG trigger day were based on evidence-based clinical protocols that have demonstrated favorable outcomes in improving endometrial function and implantation rates. These parameters were chosen to optimize the biological window of opportunity without introducing unnecessary complexity

or deviation from established clinical standards.

At baseline, a comprehensive dataset was gathered for each participant, including demographic characteristics (such as age, BMI, and infertility duration) and endocrine hormonal profiles involving FSH, LH, E2, prolactin, and thyroid-stimulating hormone (TSH), as well as transvaginal ultrasound (TVUS) examination to assess baseline endometrial thickness and antral follicle count (AFC) and to exclude any ovarian cyst or uterine pathology. All were done on cycle days two or three. In addition to structural measurements, Doppler ultrasound was used to evaluate uterine blood flow indices. These included pulsatility index (PI), resistance index (RI), and V1/V2 ratio, all of which were recorded at two distinct time points: pre- and post-G-CSF administration (Pre-G-CSF refers to the day of HCG administration or trigger day, which is when one or more dominant follicles reach around 18–22 mm in size, often on day 12 of the cycle), and Post-G-CSF (refers to IUI day). A blinding process was performed in this study to minimize bias. Both participants and outcome investigators were blinded to the route of G-CSF administration. The interventions were recorded, and all laboratory and clinical outcome assessments were performed irrespective of group allocation. Doppler ultrasound was used to assess the circulatory velocity waves from sub-endometrial arteries by positioning the entry point across the colored region in zone 2 and initiating the pulsed Doppler functionality as indicated in Equations (1) and (2) [59].

$$\mathrm{RI} = (\mathrm{PSV} - \mathrm{EDV})/\mathrm{PSV} \tag{1}$$

$$\mathrm{PI} = (\mathrm{PSV} - \mathrm{EDV})/\mathrm{MV}, \text{ where } \mathrm{MV} = (\mathrm{PSV} + \mathrm{EDV})/2 \tag{2}$$

Where:
RI = resistance index; PSV = peak systolic velocity; EDV = end-diastolic velocity; PI = pulsatility index; mv = mean velocity.

Furthermore, serum samples were also collected pre- and post-G-CSF administration (on trigger and IUI days) to measure serum levels of key immunological and angiogenic biomarkers: TNF-α, IL-10, and VEGF. Blood was drawn using a sterile syringe, allowed to clot at 37°C for 30 minutes, and spun at 1000 rpm for 10 minutes. The serum was preserved at −20°C till analyses [60,61]. Male partners of the participants were evaluated at the male infertility clinic. Their evaluation involved a detailed medical history, a physical examination including both general and scrotal assessments, and a seminal fluid analysis (SFA), carried out in accordance with the World Health Organization (WHO) guidelines published in 2010. For cases identified as mild male factor infertility, a repeat semen analysis was obtained before the IUI cycle. The final post-wash total motile sperm count used for insemination was within the range of 5–10 x 10^6 spermatozoa [62].

2.3. Biomarker Analysis

TNF-α, IL-10, and VEGF levels were estimated employing a sandwich ELISA based on biotin double-antibody technology. Wells pre-coated with specific monoclonal antibodies captured the target antigen, followed by incubation with biotin-labeled antibodies and streptavidin-HRP to form an immune complex [63–65]. After washing, a substrate was added to produce a color change proportional to the cytokine concentration [66–68]. This method was applied uniformly to all three biomarkers. The detectable range for TNF-α is 3 ng/L to 900 ng/L, with a sensitivity of 1.52 ng/L. The detectable range for IL-10 was between 2 ng/L and 600 ng/L with a sensitivity of 1.04 ng/L. The detectable range of VEGF was between 20 ng/L and 6000 ng/L, with a sensitivity of 10.42 ng/L. Intra-Assay: CV < 8%; Inter-Assay: < 10%.

2.4. Intrauterine Insemination (IUI) Cycle

Fertility drugs were employed to induce ovulation by supporting the maturation of two to three follicles. Letrozole was administered orally twice daily for five days, starting on cycle day two or three [69]. A single-dose prefilled syringe injection of Follisurge ampule 75 IU (recombinant human follicle-stimulating hormone, INTAS, India) was administered; the number of injections was adjusted according to the ovarian response, evaluated via transvaginal ultrasound [70], as it was employed to assess the antral follicle count (AFC) and endometrial thickness commencing on cycle day 2, followed by assessments on cycle day 8 and every other day thereafter to adjust medication dosages based on the patient's needs to avoid ovarian hyper-stimulation syndrome (OHSS) and multiple gestations.

On the trigger day (i.e., on the day of HCG administration to induce ovulation when one or more dominant follicles reach about 18–22 mm in size) and before HCG injection, estimation of the size and number of developing follicles, evaluation of endometrial thickness and checking the vascularity of subendometrial blood vessels via Doppler ultrasound were conducted. Then, the patients received an injection of HCG (Coriosurge XP Chorionic Gonadotrophin Vial, 5000 IU) to stimulate ovulation when ultrasound assessments on cycle days 11, 12, or 13 indicated the maturation of one to three follicles. In this study, granulocyte colony-stimulating factor (G-CSF) was given on the trigger day. G-CSF was given to interventional groups by intrauterine infusion in group 1 and by subcutaneous injection in group 2, while the control group did not receive a G-CSF injection. Semen was prepared by separating spermatozoa from seminal plasma using either a direct swim-up technique without centrifugation (simple layering technique) or a direct swim-up technique with centrifugation. The media that was used in semen preparation was Sperm Prime Media. Then the insemination catheter was connected to the 1 mL syringe containing the processed sample, and the cannula was inserted gently via the cervical canal. The sperm portion was thereafter evacuated with care, and the cannula was carefully retracted. The patient was maintained in a supine position for 30 minutes following insemination.

2.5. Statistical Analysis

Data analysis was performed using SPSS version 23.0 and Microsoft Office 2010. Descriptive statistics (mean, frequency, standard error) were used to summarize the data. ANOVA was applied for multiple group comparisons, independent t-tests for between-group analysis, paired t-tests for within-group comparisons, and chi-square tests for categorical data. A p-value ≤ 0.05 was considered statistically significant [71,72].

3. Results

All demographic and clinical data are presented as mean ± standard error of the mean (SE). No statistically significant differences were observed between the three groups in terms of age (p = 0.689), body mass index (BMI; p = 0.430), or duration of infertility (p = 0.540). Similarly, no substantial group differences were found concerning the type (p = 0.407) or causes of infertility (p = 0.483) (**Table 1**). Baseline hormonal assessments revealed no significant differences among the three groups for FSH, LH, estradiol, prolactin, and TSH, indicating a comparable endocrine status prior to intervention (**Table 2**).

Before G-CSF administration, no considerable variations were recorded between groups regarding endometrial thickness, pulsatility index (PI), resistance index, or V1/V2 ratio. Significant enhancements in endometrial thickness, PI, RI, and V1/V2 ratio were observed in both infusion and subcutaneous groups post-G-CSF (all p < 0.05), suggesting improved endometrial receptivity. In contrast, the control group exhibited increased endometrial thickness alone (p < 0.001) without significant changes in vascular indices (**Table 3**). Post hoc analysis indicated significant improvements in these vascular indices in both G-CSF groups compared to the control, but not between the G-CSF groups themselves (**Table 4**).

No significant intergroup differences were detected in baseline levels of Tumor necrosis factor-alpha (TNF-α; p = 0.404), vascular endothelial growth factor (VEGF; p = 0.995), or interleukin-10 (IL-10; p = 0.234) (**Table 5** and **Figure 1**). Post-intervention analysis similarly revealed no major variations across the three groups in serum TNF-α (p = 0.854), VEGF (p = 0.235), or IL-10 (p = 0.936) levels (**Table 6** and **Figure 2**). Following G-CSF administration, significant reductions in TNF-α (p < 0.001) and significant increases in VEGF were observed across all groups. IL-10 concentrations increased significantly in both the subcutaneous and control groups, whereas the increase in the infusion group was not statistically significant (**Table 7**). Seven infertile females out of 75 females became pregnant (overall pregnancy rate = 9.3%) (**Figure 3**). The pregnancy rates among the infusion, subcutaneous, and control groups were 16.0%, 8.0%, and 4.0%, respectively. However, there were no significant differences in pregnancy rates among the 3 studied groups (p = 0.332). There were also no significant differences between the combined interventional groups and the control group (p = 0.071) or between the infusion and control groups (p = 0.157), as illustrated in **Table 8**.

Table 1. Comparison of demographic features among infusion, subcutaneous, and control groups.

Demographic Features		Infusion Group N. = 25	Subcutaneous Group N. = 25	Control Group N. = 25	*p* Value
Age (Years) (Mean±SE)		28.12 ± 1.20	29.48 ± 1.09	29.05 ± 1.10	0.689 ∀ NS
BMI (Kg/m^2) (Mean±SE)		27.39 ± 0.91	27.40 ± 0.66	28.66 ± 0.78	0.430 ∀ NS
Duration of Infertility (Years) (Mean±SE)		2.84 ± 0.39	3.44 ± 0.46	2.88 ± 0.42	0.540 ∀ NS
Type of Infertility N. (%)	Primary	7 (28.0 %)	11 (44.0 %)	11 (44.0 %)	0.407∈ NS
	Secondary	18 (72.0 %)	14 (56.0 %)	14 (56.0 %)	
Causes of Infertility N. (%)	Female causes	14 (56.0 %)	15 (60.0 %)	13 (52.0 %)	0.483 ∈ NS
	Male causes	2 (8.0 %)	5 (20.0 %)	7 (28.0 %)	
	Combined causes	1 (4.0 %)	2 (8.0 %)	0 (0.0 %)	
	Unexplained causes	8 (32.0 %)	3 (12.0 %)	5 (20.0 %)	

Table 2. Comparison of basal hormonal levels among infusion, subcutaneous, and control groups.

Hormones (Mean±SE)	Infusion Group N. = 25	Subcutaneous Group N. = 25	Control Group N. = 25	*p* Value
FSH (mIU/ml)	6.70 ± 0.53	8.67 ± 1.29	6.54 ± 0.39	0.127 ∀
LH (mIU/ml)	5.60 ± 0.62	5.68 ± 0.72	7.56 ± 1.39	0.284 ∀
Basal E2 (pg/ml)	42.37 ± 5.71	41.73 ± 5.66	67.68 ± 6.15	0.139 ∀
Prolactin (ng/ml)	19.40 ± 1.32	18.31 ± 1.63	20.40 ± 2.11	0.700 ∀
TSH (mIU/ml)	1.92 ± 0.22	1.81 ± 0.20	1.65 ± 0.11	0.561 ∀

Table 3. Comparison of ultrasound parameters among studied groups at days of trigger and IUI (before and after G-CSF administration).

Ultrasound _Doppler Parameters	Pre-GCSF (Mean ± SE)	Post-GCSF (Mean ± SE)	*p* Value
Infusion Group			
Endometrial Thickness	8.19 ± 0.25	9.41 ± 0.31	< 0.001 ℙS
Pulsatility Index	0.73 ± 0.03	0.59 ± 0.02	0.001 ℙS
Resistance Index	0.53 ± 0.02	0.45 ± 0.01	0.001 ℙS
V1/V2 Ratio	1.89 ± 0.10	2.09 ± 0.03	0.002 ℙS
Subcutaneous Group			
Endometrial Thickness	8.70 ± 0.38	9.58 ± 0.38	0.005 ℙS
Pulsatility Index	0.73 ± 0.03	0.63 ± 0.03	0.003 ℙS
Resistance Index	0.53 ± 0.02	0.47 ± 0.02	0.012 ℙS
V1/V2 Ratio	1.90 ± 0.07	1.98 ± 0.06	0.002 ℙS
Control Group			
Endometrial Thickness	8.46 ± 0.33	9.65 ± 0.36	< 0.001 ℙS
Pulsatility Index	0.73 ± 0.02	0.72 ± 0.02	0.366 ℙNS
Resistance Index	0.52 ± 0.01	0.54 ± 0.01	0.323 ℙNS
V1/V2 Ratio	1.93 ± 0.10	1.85 ± 0.06	0.057 ℙNS

Table 4. Post hoc tukey test for paired groups' interaction of ultrasound parameters among studied groups at day of IUI (after G-CSF administration).

Ultrasound _Doppler	Paired group interaction		*p* Value
Pulsatility Index	Infusion group	Control group	0.001 S
	Subcutaneous group	Control group	0.017 S
	Infusion group	Subcutaneous group	0.519 NS
Resistance Index	Infusion group	Control group	< 0.001 S
	Subcutaneous group	Control group	0.007 S
	Infusion group	Subcutaneous group	0.666 NS
V1/V2 Ratio	Infusion group	Control group	< 0.001 S
	Subcutaneous group	Control group	0.020 S
	Infusion group	Subcutaneous group	0.432 NS

Note: S: Significant (p ≤ 0.05); Ns: Not Significant (p > 0.05); V1/V2 Ratio: Peak systolic/ end diastolic ratio.

Table 5. Comparison of biomarkers (TNF-α, VEGF, IL-10) among the studied groups at the day of trigger (before G-CSF administration).

Parameters (Mean ± SE)	Infusion Group N. = 25	Subcutaneous Group N. = 25	Control Group N. = 25	*p* Value
TNF	471.54 ± 29.2	440.40 ± 28.5	407.5 ± 37.1	0.404 ∀ NS
VEGF	1061 ± 187	1038 ± 131	1049 ± 148	0.995 ∀ NS
IL-10	46.58 ± 16.65	52.14 ± 15.38	24.24 ± 5.56	0.234 ∀ NS

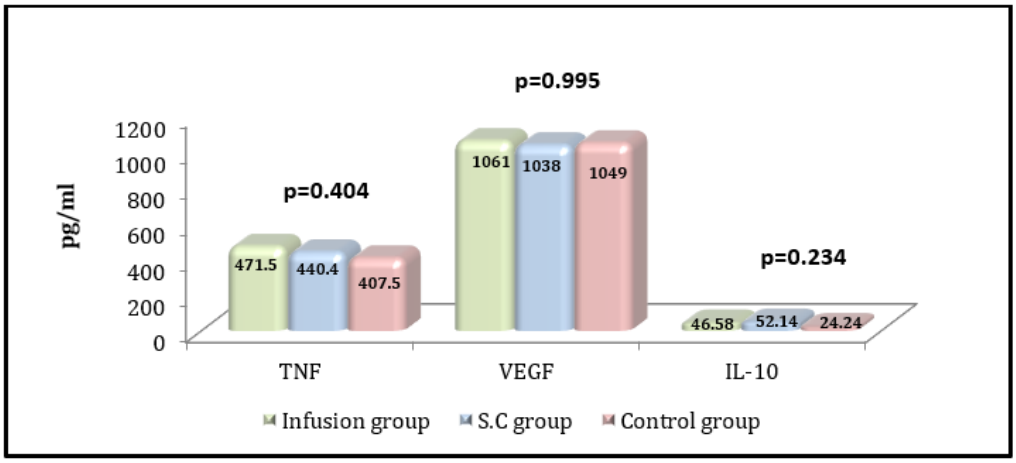

Figure 1. Graphs showing changes in biomarkers (TNF-α, VEGF, IL-10) more visually and numerically among the studied groups at the day of trigger (before G-CSF administration).

Table 6. Comparison of biomarkers (TNF-α, VEGF, IL-10) among the studied groups at the day of IUI (after G-CSF administration).

Parameters (Mean ± SE)	Infusion Group N. = 25	Subcutaneous Group N. = 25	Control Group N. = 25	*p* Value
TNF	171.87 ± 38.2	191.98 ± 34.2	198.40 ± 30.2	0.854 ∀ NS
VEGF	2572 ± 177	2392 ± 81	2375 ± 62	0.235 ∀ NS
IL-10	71.85 ± 20.57	77.42 ± 12.64	69.61 ± 15.49	0.936 ∀ NS

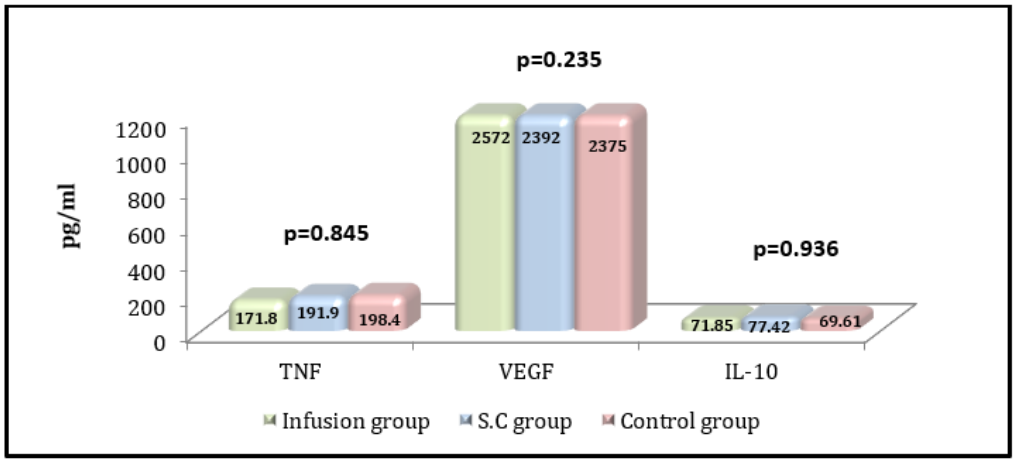

Figure 2. Graphs showing changes in biomarkers (TNF-α, VEGF, IL-10) more visually and numerically among the studied groups at the day of IUI (after G-CSF administration).

Table 7. Comparison of serum TNF-α, VEGF and IL-10 among infusion, subcutaneous, and control groups at days of trigger and IUI (before and after G-CSF administration).

Parameters (Mean ± SE)	Pre-G-CSF	Post G-CSF	*p* Value
Infusion group			
TNF	471.54 ± 29.2	171.87 ± 38.2	< 0.001 ℙS
VEGF	1061 ± 187	2572 ± 177	0.009 ℙS
IL-10	46.58 ± 16.65	59.20 ± 15.51	0.055 ℙNS

Table 7. *Cont.*

Parameters (Mean ± SE)	Pre-G-CSF	Post G-CSF	*p* Value
Subcutaneous group			
TNF	440.40 ± 28.5	191.98 ± 34.2	< 0.001 PS
VEGF	1038 ± 131	2392 ± 81	< 0.001 S
IL-10	52.14 ± 15.38	77.42 ± 12.64	< 0.001 PS
Control group			
TNF	407.5 ± 37.1	198.40 ± 30.2	< 0.001 PS
VEGF	1049 ± 148	2375 ± 62	0.002 PS
IL-10	24.24 ± 5.56	48.82 ± 4.70	< 0.001 PS

Table 8. Comparison of pregnancy rate among the studied groups.

	Infusion Group	Subcutaneous Group	Control Group	*p* Value
Pregnancy Rate	4/25 (16.0%) Interventional groups N. = 50 6/50 (13.6%) Infusion group N. = 25 4/25 (16.0%)	2/25 (8.0%)	1/25 (4.0%) Control group N. = 25 1/25 (4.0%) Subcutaneous group N. = 25 2/25 (8.0%)	0.332 Ɛ NS *p* value 0.071 Ɛ NS *p* value 0.157 Ɛ NS

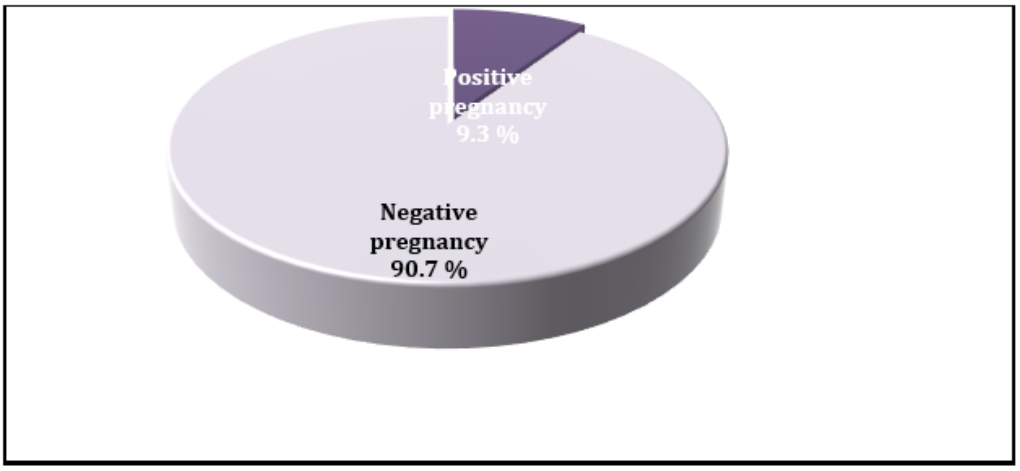

Figure 3. Pregnancy rate of patients enrolled in the present study.

4. Discussion

This study investigated whether intrauterine versus subcutaneous application of G-CSF could strengthen endometrial receptivity by modifying vascular and immune markers in infertile women undergoing IUI. The results indicate that both administration routes improved endometrial perfusion, as evidenced by significant reductions in pulsatility index (PI), resistance index (RI), and increased V1/V2 ratio, particularly in the intrauterine group.

Immunologically, G-CSF administration was correlated with a substantial reduction in serum TNF-α amounts and a notable increment in VEGF levels across all groups. Cytokines were once thought to be critical regulators for immune system homeostasis [73–75]. They may encourage or hinder cell development, affect cellular differentiation, initiate cell mobility, and influence the creation of additional signaling molecules and pathways [76–79]. TNF-α is a pro-inflammatory cytokine [80] that, in excessive amounts, is implicated in implantation failure and endometrial dysfunction [81]. The observed decrease suggests an anti-inflammatory shift conducive to implantation. During implantation, the mother's immune system is challenged. By altering the cytokine profiles of T cells, GCSF encourages Th2 responses, the growth of tolerant dendritic cells, and the production of IL-10-producing T regulatory cells. Crucial immune regulatory phases occur both before and after implantation in the uterus. Although IL-10, an anti-inflammatory cytokine [82,83], increased significantly only in the subcutaneous and control groups, the near-significant increase in the infusion group ($p = 0.055$) indicates a potential immunomodulatory benefit that

requires larger sample sizes to validate. A noteworthy finding was the increase in IL-10 in the control group, which may suggest a physiological fluctuation during the luteal phase or an indirect consequence of the IUI procedure itself.

IUI has been proposed as a familiar option for treating infertility [84]. It is often employed in couples experiencing modest male-related infertility, unidentified infertility, minor endometriosis, cervical aspects, and psychiatric sexual problems. Implantation is considered a crucial stage of pregnancy [85,86].

Body mass index (BMI), a recognized predictor of reproductive outcomes, was likewise comparable across all groups in the current study, reaffirming its longstanding detrimental influence on fertility in obese women [87]. In addition to demographic variables, baseline hormonal profiles were analyzed and found to be statistically comparable across all groups. There were no significant differences in serum levels of FSH, LH, estradiol (E_2), prolactin, or TSH (all $p > 0.05$). This aligns with recent studies, which showed no significant differences in hormonal profiles among infertile women, as all patients were selected within the same inclusion and exclusion criteria, which exclude women with hormonal or systemic disorders [88].

Cytokines can regulate cellular multiplication, immune tolerance, and the establishment of circumstances necessary for fetal growth, differentiation, and function [89,90]. Pregnancy presents an immunological challenge to the mother due to the semi-allogenic origin of the fetus [91,92]. The current study findings demonstrated that both routes of G-CSF administration positively influence endometrial vascularity, particularly in the infusion group, and immune biomarkers associated with implantation. However, the impact on pregnancy outcomes was not statistically significant. The significant improvements observed in Doppler ultrasound parameters—namely, the reduction in pulsatility index (PI) and resistance index (RI), along with the increase in the V1/V2 ratio—in both G-CSF treatment groups, particularly in the infusion group, suggest an enhancement in endometrial perfusion. These results align with prior evidence indicating that G-CSF contributes to vascular remodeling and angiogenesis in the endometrium by amplifying VEGF [93,94]. These hemodynamic improvements are consistent with the proposed role of G-CSF in enhancing endometrial receptivity by modulating angiogenesis, as seen in a study that shows significantly higher endometrial vascularity in the G-CSF group on the day of embryo transfer [95]. Post hoc analysis confirmed that both the infusion and subcutaneous groups demonstrated significantly better PI, RI, and V1/V2 ratios than the control group, with no statistically significant difference between the infusion and subcutaneous groups. These findings suggest that both methods of G-CSF administration are effective, although infusion may yield a slightly superior perfusion profile. In contrast, another study related the improvement in tissue remodeling and angiogenesis to G-CSF injection administered to women who reached the day of embryo transfer via the subcutaneous route [96]. One study demonstrated that intrauterine G-CSF infusion significantly reduced uterine artery resistance in women with recurrent implantation failure [97], a finding that parallels the vascular changes observed in the current study. However, Check et al. (2021) found no significant changes in uterine blood flow parameters after G-CSF administration in a cohort undergoing IVF, suggesting that the impact may vary by route of administration, baseline endometrial receptivity, and the assisted reproduction technique used [98].

Moreover, the elevation in VEGF reinforces the proposed angiogenic role of G-CSF. Although both intervention groups demonstrated elevated post-treatment serum VEGF levels compared to baseline, the absence of significant intergroup differences in VEGF concentrations suggests a ceiling effect or systemic saturation (this implies that there may be a biological limit to how much VEGF the body can produce or respond to). So even if the treatments were different, they both hit that upper limit. Nonetheless, the highest mean increase in VEGF was observed in the intrauterine infusion group, consistent with the localized paracrine enhancement of the endometrial microenvironment. Similarly, G-CSF may exert its effects by modulating the endometrial cytokine and growth factor environment, thereby contributing to improved microcirculation [99]. The improved vascular indices, without a concomitant increase in endometrial thickness, emphasize that perfusion quality may be a more reliable predictor of endometrial receptivity than thickness alone [100]. A significant increase in endometrial thickness was limited to women with a thin endometrium receiving G-CSF, with <7 mm thickness at baseline [101]. These findings sympathize with those who took one hundred twenty infertile women aged 20–40 years undergoing IUI and suggested that G-CSF, compared to placebo, improved endometrial thickness in patients with thin endometrium in IUI cycles. However, the improvement was not statistically significant [102]. Current study participants had normal baseline endometrial thickness (≥ 8 mm), which may explain the lack of effect. This supports the hypothesis that G-CSF is more beneficial in cases of endometrial insufficiency rather than normo-thick endometrium [103,104].

It is important to recognize that VEGF plays a crucial role in regulating angiogenesis and endometrial vascular remodeling, two processes that naturally fluctuate during the menstrual cycle, particularly in the luteal phase following ovulation (from the trigger day to the IUI day). The observed increase in VEGF in the control group may therefore reflect a normal physiological response to hormonal changes and endometrial preparation for potential implantation, independent of exogenous G-CSF administration [105]. Additionally, IUI itself may induce local endometrial changes, including mild inflammatory and repair responses, which can stimulate VEGF expression as part of the tissue remodeling and vascular adaptation process. Such procedural effects have been documented in reproductive studies showing that uterine manipulation can transiently influence cytokine profiles and angiogenic factors. Thus, while G-CSF administration appears to augment further VEGF levels—consistent with its established role in promoting angiogenesis—this study's data suggest that endogenous mechanisms linked to the menstrual cycle and/or the IUI procedure may also drive VEGF increases. This may explain the significant elevation of VEGF observed across all groups, including controls [106].

Interestingly, the magnitude and associated vascular improvements (e.g., Doppler indices) were more pronounced in the G-CSF-treated groups, especially with intrauterine infusion, supporting a synergistic or enhancing effect of G-CSF beyond physiological baseline changes [107]. Ultimately, the increase in VEGF in the control group does not contradict the role of G-CSF, but rather highlights the complexity of endometrial angiogenesis regulation, which involves both intrinsic cyclical factors and external interventions. Future studies incorporating placebo controls and more frequent longitudinal sampling could further dissect these dynamics.

However, without a placebo-controlled design, such interpretations remain speculative. The lack of significant differences in hormonal profiles and baseline endometrial and immunological parameters across groups confirms that the observed post-intervention changes are likely attributable to the administration of G-CSF. Moreover, the comparable efficacy between intrauterine and subcutaneous routes in improving endometrial perfusion suggests that systemic and local administration may both exert meaningful biological effects, albeit through potentially different mechanisms or pharmacokinetics [108]. The local intrauterine route may offer a more targeted approach with fewer systemic effects; however, this requires a direct pharmacodynamic comparison. This work has numerous drawbacks. The sample size, although sufficient for detecting vascular and immunological changes, may not be adequately powered to examine distinctions in clinical pregnancy rates, despite the higher pregnancy rate in the G-CSF groups, especially in the infusion group, although this difference is not statistically significant. Furthermore, the study did not assess long-term outcomes such as live birth rates or potential adverse effects of G-CSF. Future trials with larger cohorts, extended follow-up, and possibly histological confirmation of endometrial receptivity would offer a more complete picture of G-CSF's therapeutic value.

It is also noteworthy that G-CSF influences uterine natural killer (uNK) cell populations, which play a central role in regulating maternal immune tolerance during early pregnancy. Several studies have reported that G-CSF can modulate uNK cell activity, enhancing their angiogenic profile while reducing cytotoxicity, thereby creating a microenvironment that is favorable for implantation [109]. These findings are congruent with the current study's observed changes in immunological markers such as TNF-α and IL-10. By promoting a shift from a pro-inflammatory Th1-dominant milieu to a more tolerant Th2 or Treg-enriched profile, G-CSF potentially mitigates subclinical endometrial inflammation, a recognized barrier to implantation success.

Additionally, angiogenic and immune pathways are often intricately linked. G-CSF may serve as a convergence point between these domains by fostering cross-talk between endothelial cells and immune mediators. For instance, the observed VEGF elevation likely interacts with nitric oxide (NO) pathways, which play key roles in vasodilation and vascular permeability essential for successful embryo implantation [110]. Although this study did not directly assess NO metabolites, it may be beneficial for future investigations to incorporate these endpoints to understand the downstream vascular implications of G-CSF better.

Moreover, while pregnancy outcomes were not significantly different among groups, the trend toward improved rates in the intrauterine group is clinically promising and should not be overlooked. According to recent meta-analyses, even small improvements in endometrial receptivity biomarkers, such as uterine artery PI and RI, correlate with higher implantation and clinical pregnancy rates in IUI and IVF cycles [111]. Therefore, G-CSF's potential lies not only in improving vascular and immune parameters but also in subtly tipping the balance toward a receptive phenotype, especially in borderline or functionally inadequate endometrial conditions.

An additional point to consider is the pharmacological rationale supporting intrauterine versus systemic G-CSF

administration. While subcutaneous administration offers ease and systemic distribution, it may result in lower local bioavailability within the endometrium. Conversely, intrauterine infusion delivers a concentrated dose directly to the target site, maximizing paracrine and autocrine signaling benefits while potentially reducing systemic exposure and side effects [112]. Pharmacokinetic studies comparing serum and endometrial fluid G-CSF levels following both administration routes could elucidate these dynamics more precisely.

Reproductive applications of G-CSF require rigorous safety evaluations, especially concerning long-term maternal and offspring outcomes. Some studies suggest that repeated or high-dose G-CSF exposure may be associated with epigenetic changes in placental tissues; however, the data remain inconclusive [113]. Therefore, as its use expands in fertility treatments, longitudinal safety studies and post-marketing surveillance are imperative.

In terms of clinical applicability, G-CSF may be especially valuable for certain patient subsets, such as those with repeated implantation failure (RIF), a thin endometrium, or endometrial immune dysregulation. Personalized medicine approaches incorporating immune and angiogenic biomarker screening could help identify patients most likely to benefit from G-CSF therapy [114]. This aligns with the growing emphasis on tailoring fertility interventions based on individual endometrial receptivity profiles, as seen with the endometrial receptivity array (ERA) and other transcriptomic tools [115].

Lastly, the utility of combining G-CSF with other adjuncts such as platelet-rich plasma (PRP), sildenafil citrate, or low-dose aspirin may offer synergistic benefits. Some studies have demonstrated that combination therapy may further amplify endometrial angiogenesis and reduce inflammatory mediators, thereby enhancing the implantation niche [116]. Though this was beyond the scope of the current study, future trials could explore such multimodal strategies to optimize outcomes.

5. Conclusion

This study provides evidence that both intrauterine and subcutaneous G-CSF administration can enhance endometrial receptivity through vascular and immunological modulation, specifically for unexplained infertility. While the intrauterine route showed slightly more favorable outcomes, further trials are essential to determine the clinical effectiveness and patient-specific applicability. Clinically, G-CSF can be added as an adjuvant treatment for future ART protocols.

6. Study Limitations

While the sample size was adequate for detecting changes in vascular and immune markers, it was underpowered to establish statistical differences in pregnancy outcomes, despite a higher (non-significant) pregnancy rate in the G-CSF groups, especially the infusion group. Furthermore, long-term consequences, such as live birth rate and potential adverse effects, were not assessed. Future randomized controlled trials with larger cohorts, placebo arms, and histological evaluation of endometrial receptivity are warranted to confirm the clinical utility and optimal administration route of G-CSF in IUI cycles.

Author Contributions

Conceptualization, Z.A.Q.M. and L.A.A.; methodology, M.T.A.; software, Z.A.Q.M.; validation, Z.A.Q.M., L.A.A., and M.T.A.; formal analysis, L.A.A.; investigation, M.T.A.; resources, Z.A.Q.M.; data curation, Z.A.Q.M., L.A.A., and M.T.A.; writing—original draft preparation, Z.A.Q.M.; writing—review and editing, Z.A.Q.M.; visualization, L.A.A.; supervision, L.A.A. and M.T.A.; project administration, M.T.A.; funding acquisition, Z.A.Q.M. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement

The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board at Al-Nahrain University, High Institute for Infertility Diagnosis and Assisted Reproductive Technologies

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Informed Consent Statement

Informed consent was obtained from all subjects involved in the study.

Data Availability Statement

The data underlying the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare no conflict of interest.

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