Retrospective Review ISSN 2639-9342

Gynecology & Reproductive Health

# Comparison of Adjunctive Use of G-CSF Vs Autologous PRP in IVF Patients with a Refractory Thin Endometrium: A Retrospective Record Review

**Mohamed Iqbal Cassim[1], Tasneem Mohamed[1*], Yusuf Mohamed Dasoo[1], Justin Carlse[2], Simoné Budler[2], Erwin Rathipal[2], Muhammad Ayob[2], Jeanne Saron Nissieh Sokoni[2], Suhail Dudhia[2] and Mohamed Fayaaz Cassim[2]**

[1]*BioART Fertility Clinic, Johannesburg, South Africa.*

[2]*University of Witwatersrand, Johannesburg, South Africa.*

**[*]Correspondence:**
Dr. Tasneem Mohamed, BioART Fertility Clinic, Johannesburg, South Africa, 99 Oxford Road, Saxonwold, Johannesburg 2196, South Africa.

**Received:** 01 Nov 2022; **Accepted:** 02 Dec 2022; **Published:** 07 Dec 2022

**Citation:** Cassim MI, Mohamed T, Dasoo YM, et al. Comparison of Adjunctive Use of G-CSF Vs Autologous PRP in IVF Patients with a Refractory Thin Endometrium: A Retrospective Record Review. Gynecol Reprod Health. 2022; 6(6): 1-9.

## ABSTRACT

***Background:*** *In Vitro Fertilization (IVF) is an important treatment option in the management of couples with infertility. Sadly, however, failure to achieve a pregnancy through IVF is not uncommon. Amongst the many causes of IVF failure, implantation failure has emerged as one of the more common and important factors. The refractory thin endometrium as a cause of recurrent IVF failure has been well documented. The use of either Granulocyte Colony Stimulating Factor (G-CSF) or autologous Platelet Rich Plasma (PRP) has emerged as potential adjunctive treatments that may mitigate the rate of implantation failure; however, no conclusive evidence exists to favour the use of one over the other.*

***Objective:*** *To compare the measured change in endometrial thickness and pregnancy rates in patients with Recurrent implantation Failure (RIF) and/or thin endometrium following the intrauterine administration of either G-CSF or autologous PRP. In addition, to compare the pregnancy rates in patients with fluid in the endometrial cavity who underwent either therapy.*

***Design:*** *A retrospective analysis was conducted on patients who underwent endometrial therapy (either G-CSF or PRP) between January and June 2020. The measured change in endometrial thickness and the clinical pregnancy outcome of the two groups were compared.*

***Subjects:*** *36 patients with a mean age of 40.36 years met the inclusion criteria of the study. 20 received autologous intrauterine PRP treatment and 16 received intrauterine G-CSF treatment. Both groups were well matched for age, pre-intervention endometrial thickness and embryo quality.*

***Intervention:*** *Administration of G-CSF (One ampoule Neupogen® (filgrastim)) or PRP (1ml) into the uterine cavity transcervical 48 hours prior to embryo transfer.*

***Main Outcome Measures:*** *The change in endometrial thickness measured 48 hours prior to embryo transfer and at the time of embryo transfer (ET) was compared. Positive clinical pregnancy outcome was determined by a positive serum B-HCG test 10 days post insertion. A statistically significant difference was set at p=0.05.*

***Results:*** *There was a statistically significant difference in endometrial expansion post intervention in both the G-CSF and PRP groups. However, the difference between the two groups did not reach statistical significance (p=0.077). Additionally, the collective pregnancy rate of the total study population was 44.4% (16 of 36), a significant increase over the expected pregnancy rate in the published literature [1,2]. Of the positive pregnancies, 9 (56.25%) were in the autologous PRP group and 7 (43.75%) in the G-CSF group. This difference was, however, determined not to be statistically significant (p=0.603).*

***Conclusion:*** *Both G-CSF and PRP are effective interventions in the management of the thin refractory endometrium. Both result in significant endometrial expansion and increased pregnancy rates. Despite a marginally higher endometrial response and pregnancy rate in the PRP group, the differences in these metrics between the two groups were not statistically significant.*

***Keywords:*** *Granulocyte Colony Stimulating Factor, Platelet Rich Plasma, thin endometrium, implantation failure, adjunctive therapy.*

## Attestation statements

The subjects in this trial have not concomitantly been involved in other randomized trials. Data regarding any of the subjects in the study has not been previously published unless specified. Data will be made available to the editors of the journal for review or query upon request.

## Capsule

A retrospective study to compare the adjunctive use of G-CSF vs autologous PRP in IVF patients with a refractory thin endometrium and recurrent implantation failure.

## Introduction

Endometrial factors implicated in implantation failure include an elevated or disrupted patient immune response, chronic endometritis (CE), decreased endometrial gene regulation, and suboptimal endometrial thickness [3,4]. Endometrial thickness as a predictor of prognosis in IVF has been controversial. However, recent meta-analyses have indicated that the thin endometrium does indeed negatively affect pregnancy outcomes in fresh and frozen IVF- ET cycles [5,6]. Both pregnancy and live birth rates decline progressively as endometrial thickness decreases below 8mm [6,7]. Apart from potential implications on pregnancy rates, a thin endometrium seems to also be associated with other adverse events, including miscarriages and abnormal placentation [5].

With respect to the thin endometrium, Duraijaj et al., [8] suggests that this could be due to abnormalities in the decidualization process because of compromised endometrial stromal cell secretome. Other etiologies of a thin pre-implantation endometrium include previous endometrial trauma, decreased endometrial blood flow, prolonged use of oral contraceptives and the use of estrogen receptor blockers such as clomiphene citrate [3].

Several treatment modalities have been explored for the thin-endometrium related implantation failure. They can be broadly grouped into three approaches, each exploiting different mechanisms to increase endometrial thickening and receptivity. These treatments include hormonal (estrogen, GnRH, HCG), vascular (sildenafil, aspirin, pentoxifylline, neuromuscular electrical stimulation) or growth factor therapies (G-CSF, Autologous PRP) respectively. Patients with cured endometritis had better IVF outcomes than those with chronic endometritis [9].

The aim of our study was to compare the efficacy of intrauterine administration of G-CSF to autologous PRP therapy with regards to endometrial expansion and/or improved pregnancy outcomes in women with a thin refractory endometrium. Furthermore, to investigate the effect of G-CSF and autologous PRP on pregnancy rates in patients with fluid in the endometrial cavity prior to embryo transfer.

## Materials and Methods

A retrospective analysis was conducted of patients treated by IVF at the BioART Fertility Centre in Saxonwold, Johannesburg between January 2020 and June 2020. All patients who underwent either G-CSF or PRP endometrial therapy with endometrial thickness less than 8mm were reviewed. Patients at BioART are routinely offered these adjuvant therapies for either a thin endometrium or RIF. In the case of suboptimal endometrial thickness, patients are routinely supplemented with oral estradiol up to 8mg and if still an inadequate response is noted, a transdermal estradiol patch is added (50mg Evorel). In addition, all patients with suboptimal endometrial thickness are given Sildenafil 25mg orally starting at least 4 days before anticipated ET. All patients included in this study had endometrium's refractory to our routine first-line therapeutic approach and were thus considered for intrauterine therapy with either G-CSF or PRP. No consensus has been reached regarding which intrauterine treatment approach yielded better results, and thus patients included in the study had been offered either treatment modality indiscriminately. Informed consent was obtained in all cases.

A total of 47 patients underwent endometrial therapy with either G-CSF or autologous PRP during the period of January 2020 to June 2020. 36 patients met the inclusion criteria with respect to endometrial thickness and embryo quality. 20 patients received autologous PRP, and 16 patients received G-CSF.

Endometrial thickness was measured using transvaginal sonography and was measured at the thickest part of the endometrium along the longitudinal axis. This was recorded as E1 in the data collection tool. All measurements were done 48 hours before embryo transfer. Another measurement of the endometrial thickness was then performed immediately prior to embryo transfer. This reading was recorded in the data collection tool as E2, whereafter the difference between E1 and E2 was calculated.

We considered a positive serum B-HCG test at 10 days from the embryo transfer as a chemical pregnancy and a positive pregnancy outcome. A negative serum B-HCG test at 10 days from the embryo transfer was regarded as a negative pregnancy outcome.

### Grading and Quality of embryos transferred:

The embryos to be transferred were graded on the day of embryo transfer (Annexure A).

Day 3 (66-72 hours post-insemination) embryo grading criteria included number of blastomeres, evenness of the blastomeres and the degree of fragmentation. Day 5 (114-120 hours post-insemination) embryos were graded according to the size or volume of the blastocoel cavity, inner cell mass, trophectoderm and Zona Pellucida thickness. For the purposes of this study embryo quality was further grouped as either good, intermediate, or poor quality (Annexure B). At least one good quality embryo was transferred per participant.

### Method of transvaginal G-CSF and autologous PRP administration:

Both interventions were administered at room temperature to avoid

any adverse effects such as uterine spasm or vaso-vagal responses. A semi-rigid embryo transfer catheter was used for infusion. The optimum position for catheter placement was estimated using the most recent ultrasound findings. We aimed to place the catheter tip at the mid-cavity level. One ampoule of G-CSF [Neupogen® (filgrastim) 1 ml], or autologous PRP of the same quantity was instilled into the uterine cavity, ensuring that the complete volume of fluid was discharged.

### Method of autologous PRP preparation:

8 anticoagulant tubes of peripheral venous blood were collected per patient. The vials were then immediately balanced and centrifuged at 3000 revolutions per minute for 5 minutes. From the three layers, the upper plasma layer of all vials was then removed until there was at least 0.3ml of buffy coat. The buffy coats and a little bit of the erythrocyte layer was then placed in a separate tube. This was then centrifuged for a second time at 3000 revolutions per minute for a further 5 minutes. The intermediate PRP layer was then pipetted out and, where required, supplemented with serum to make up the volume of 1ml.

## Ethics

Ethical clearance for this study was granted by the University of Witwatersrand Human Research and Ethics Committee. (No M191107).

## Data Analysis

All patient identifiable data was removed. Data was exported to Statistica version 14.0.0.15 TIBCO Software Inc. (2020). Data Science Workbench, version 14 (http://tibco.com) for data analysis. Categorical variables were described using frequencies and percentages, whereas continuous variables were described using means (with Standard deviation). Categorical variables were compared using Fisher exact test. Continuous variables were compared using student t-test and Wilcoxon Mann Whitney u- test. A difference was noted to be statistically significant if the p value was less than 0.05.

## Results

### Study Population

A total of forty-seven women underwent embryo transfer with either PRP or G-CSF between the period of 1 January 2020 to 30 June 2020. Thirty-six women met the relevant criteria and were included in the study. A total of twenty women received PRP and sixteen received G-CSF as an adjunct therapy.

**i) Age:**

The ages of the participants ranged from twenty-six to fifty-six. The mean age was 40.36 (SD +/- 6.77) and the median age was 39.50 (IQR 45.00-37.00) (Figure 1). The PRP group had a mean age of 40.45 (SD +/- 7.26) and median age of 39.00 (IQR 42.75-34.75) compared with the group treated with G-CSF who had a mean age of 40.25 (SD +/- 6.33) and median age of 39.00 (IQR 42.75-34.75) (Figures 2 and 3). The two groups were not significantly different with respect to their ages (p-value= 0.469).

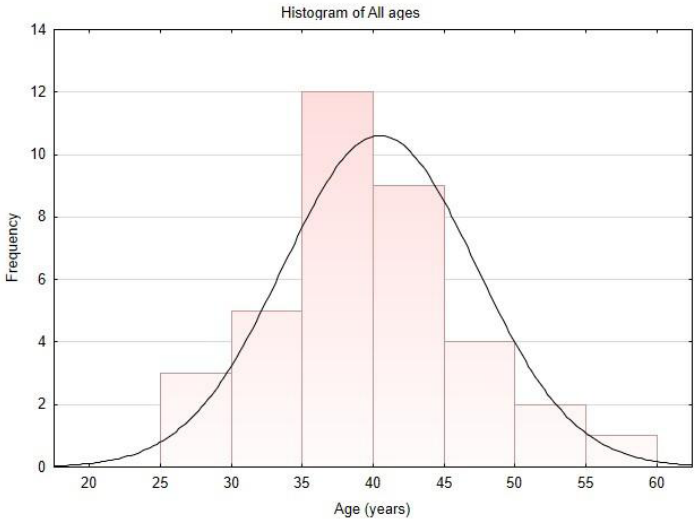


**Figure 1:** Histogram of Age Distribution of the study population.

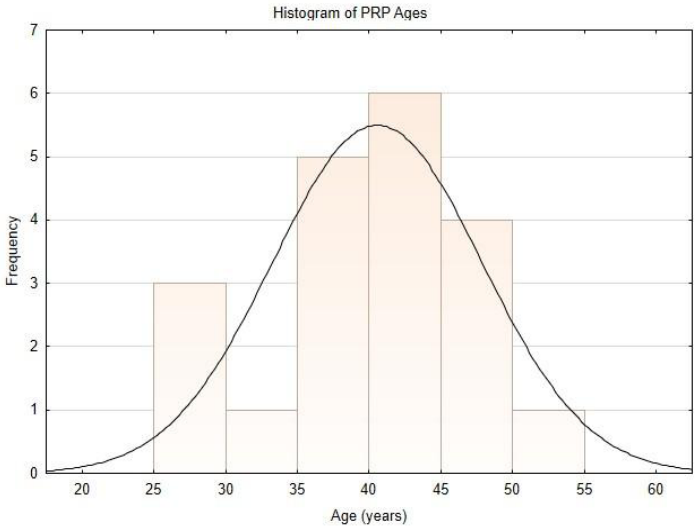


**Figure 2:** Histogram of Age Distribution in PRP group.

**ii) Smoking History:**

Only one patient had a history of being a smoker and she formed part of the PRP group. The remainder of the women in the study were life-long non-smokers. Thus, there was also no significant difference between the smoking histories of the women that received PRP and G-CSF respectively (p-value= 0.556).

**iii) BMI Classification:**

No significant difference in BMI was noted between the two groups (p-value=0.160).

**iv) HIV Status:**

A total of seven participants in the study were HIV positive, with the remaining 29 women being HIV negative. In the PRP group, five of the twenty were HIV positive (25%), with two in the G-CSF group (12.5%).

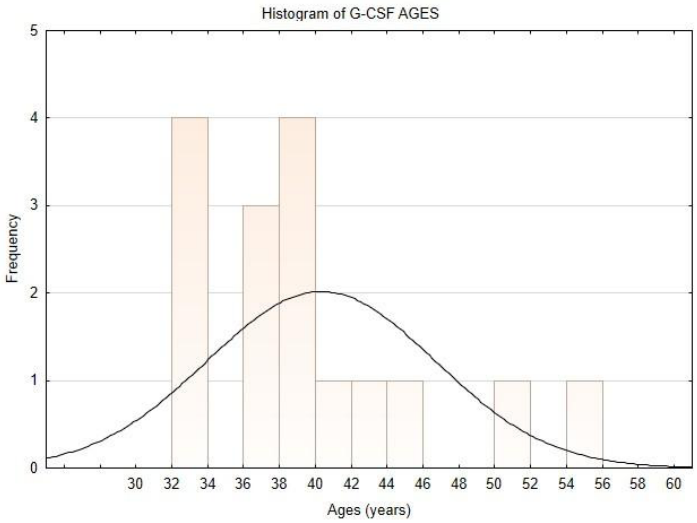


**Figure 3:** Histogram of Age Distribution in G-CSF group.

The frequency of HIV in the study population and the respective groups is indicated in Table 1 below. There was no significant difference in the two groups with respect to HIV status (p-value= 0.307).

| HIV STATUS | Autologous PRP | | G-CSF | | Total Study Population | |
|---|---|---|---|---|---|---|
| | Freq | Per | Freq | Per | Freq | Per |
| Positive | 5 | 25.00 % | 2 | 12.50 % | 7 | 19.44 % |
| Negative | 15 | 75.00 % | 14 | 87.50 % | 29 | 80.56 % |

**Table 1:** Freq: Frequency; Per: Percentage.

## Fertility History:

### i) Number of previous IVF cycles:

As indicated in Figure 4, the participants in the study had a history of between 0 to 9 previous failed attempts at IVF. The mean number of previously failed IVF cycles was 2.17 (SD +/- 1.58) and a median of 2 (IQR 1.25-0.75). The PRP group of women had a history of 0 to 6 previous failed attempts at IVF (Figure 5) with a mean of 2.1 (SD +/- 1.33) and median 2 (IQR 2.75-1.75). The G-CSF group had a history of 1 to 9 previous failed attempts at IVF (Figure 6) with a mean of 2.25 (SD +/- 1.88) and median of 2 (1.25-0.75). There is no significant difference between the number of previously failed IVF cycles in the two groups (p-value= 0.765).

### ii) Fluid present in cavity:

Table 2 indicates the frequencies of fluid in the endometrial cavity in the study population in each of the two groups. Six of the study participants had fluid in their endometrial cavity prior to the embryo transfer and of these 4 received G-CSF and 2 received PRP. There is no significant difference between the G-CSF and PRP group with regards to the presence of fluid in the endometrial cavity (p-value= 0.227).

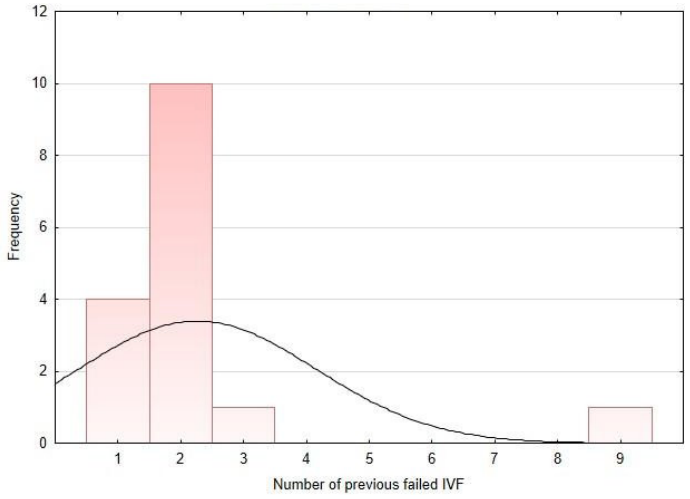


**Figure 4**: Histogram of Number of previous failed IVF cycles in the study population.

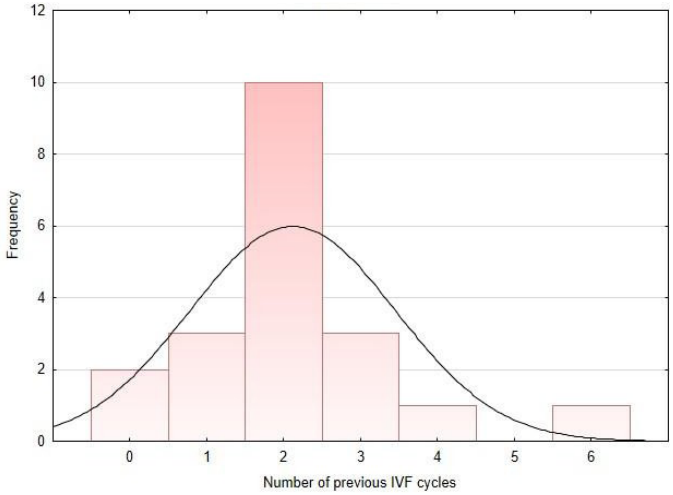


**Figure 5:** Histogram of Number of previous failed IVF cycles in the PRP group.

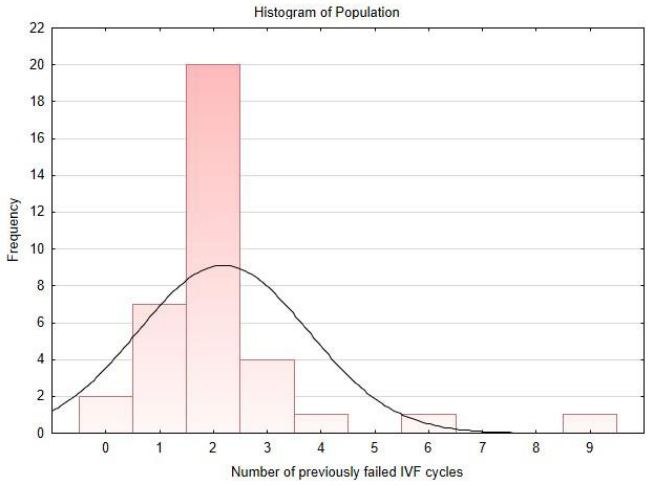


**Figure 6:** Histogram of Number of previous failed IVF cycles in the G-CSF group.

| FLUID PRESENT IN ENDO-METRIAL CAVITY | Autologous PRP | | G-CSF | | Total Study Population | |
|---|---|---|---|---|---|---|
| | Freq | Per | Freq | Per | Freq | Per |
| Present | 2 | 10.00 % | 4 | 25.00 % | 6 | 16.67 % |
| Absent | 18 | 90.00 % | 12 | 75.00 % | 30 | 83.33 % |

**Table 2:** Freq: Frequency; Per: Percentage.

## Cycle Specific Data:

### i) Number of embryos transferred:

Between 1 and 3 embryos were transferred during each transfer cycle. The mean number of embryos transferred was 2.19 (SD +/- 0.62) and median of 2 (IQR 3-2) as seen in Figure 7. The PRP group had a mean of 1.95 (SD +/- 0.61) and a median of 2 (IQR 2-2) embryos transferred (range 1 to 3). The G-CSF group had a mean of 2.50 (SD +/- 0.52) and median of 2.50 (IQR 3-2) number of embryos transferred (Range 2 to 3). Figures 8 and 9 indicate the distribution of the number of embryos transferred in the respective groups. A statistically significant difference was demonstrated between the two respective groups with regards to the number of embryos transferred (p-value= 0.009).

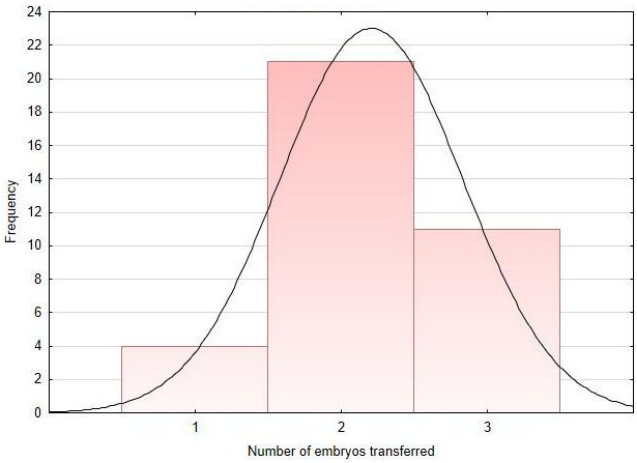


**Figure 7:** Histogram of Number of embryos transferred in the study population.

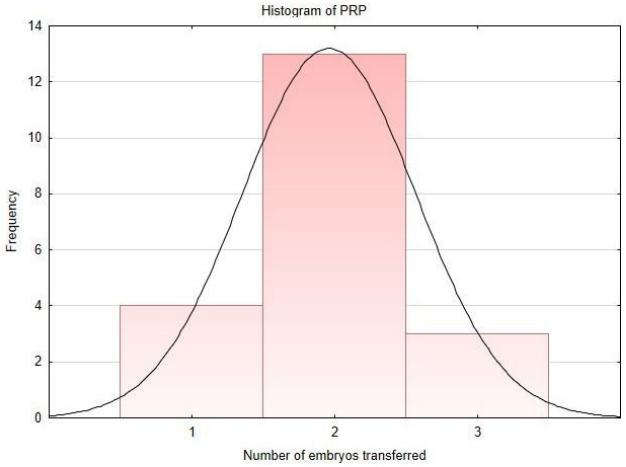


**Figure 8:** Histogram of Number of embryos transferred in the PRP group.

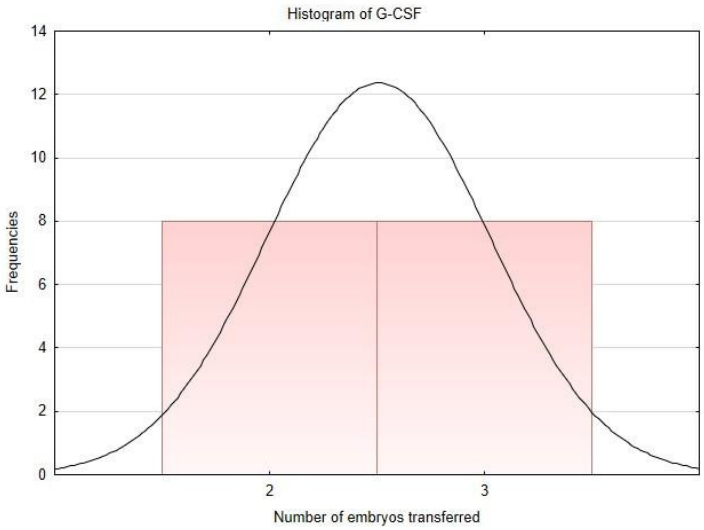


**Figure 9:** Histogram of Number of embryos transferred in the G- CSF group.

### ii) Type of embryos transferred:

Two of the participants in the G-CSF group had a fresh embryo transfer. All remaining participants had frozen embryo transfer using the GnRH protocol. Embryo frequencies are indicated in table 3 below. No statistically significant difference was noted between the two groups with regards to the type of embryos transferred (p-value= 0.078).

### iii) Grading of Embryos:

A total number of 77 embryos of varying quality were transferred during the study. For the purposes of this study, these embryos were classified as good, intermediate, and poor quality (Appendix A and Table 3). No statistically significant difference was found between the quality of embryos that were transferred to each of the respective intervention groups (p-value= 0.450).

| EMBRYO QUALITY | Autologous PRP | | G-CSF | | Total Study Population | |
|---|---|---|---|---|---|---|
| | Freq | Per | Freq | Per | Freq | Per |
| Good | 28 | 73.68 % | 23 | 58.97 % | 51 | 64.94 % |
| Intermediate | 10 | 26.32 % | 15 | 38.46 % | 25 | 24.68 % |
| Poor | 0 | 0.00 % | 1 | 2.56 % | 1 | 0.00 % |

**Table 3:** Freq: Frequency; Per: Percentage.

## Change in Endometrial thickness

Participants in the autologous PRP group had an endometrial thickness ranging from 1.00 mm to 7.60 mm prior to receiving PRP. The mean endometrial thickness was 6.58 mm (SD +/- 1.56 mm) prior to the intervention. After PRP insertion, the endometrial thickness ranged from 5.90 mm to 10.70 mm with a mean of 7.98 (SD +/- 1.41 mm). Comparison of the endometrial thickness prior to PRP and after PRP administration using a paired t-test shows a mean expansion of 1.41 mm with 95% Confidence Interval (0.87-1.94) and is statistically significant with p<0.0001 (Figure 10 and Table 4).

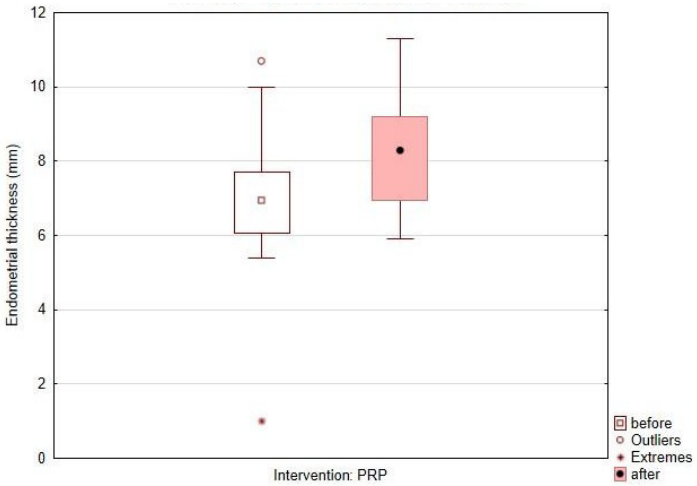


**Figure 10:** Boxplot of Endometrial thickness prior to and after autologous PRP administration.

Paired t test

| Variable | Obs | Mean | Std. Err. | Std. Dev. | [95% Conf Interval] |
|---|---|---|---|---|---|
| xnum_e1 | 20 | 6.575 | 0.3479924 | 1.556269 | 5.846644 - 7.303356 |
| xnum_e2 | 20 | 7.98 | 0.3154445 | 1.410711 | 7.319767 - 8.640233 |
| diff | 20 | -1.405 | 0.2577713 | 1.152788 | -1.944522 - 0.8654784 |

Mean (diff) = mean (xnum_e1 – xnum_e2) t = -5.4506
Ho: mean (diff) =0 degrees of freedom = 19
Ha: mean (diff) < 0 Ha: mean (diff) !=0 Ha: mean (diff) > 0
Pr (T < t) = 0.0000 Pr ( |T| > |t| ) = 0.0000 Pr (T > t) = 1.0000

**Table 4**

The G-CSF group had an endometrial thickness ranging from 3.10 mm to 7.80 mm prior to intervention, with a mean endometrial thickness of 6.56 mm (SD +/- 2.33 mm). After receiving G-CSF, the endometrial thickness ranged from 5.9 mm to 10.80 mm with a mean of 7.50 (SD +/- 2.22 mm). Comparison of the endometrial thickness prior to and post G-CSF insertion using a paired t-test shows a mean expansion of 0.94mm with 95% Confidence Interval (0.29-1.59). This is statistically significant with p=0.007 (Figure 11 and Table 5).

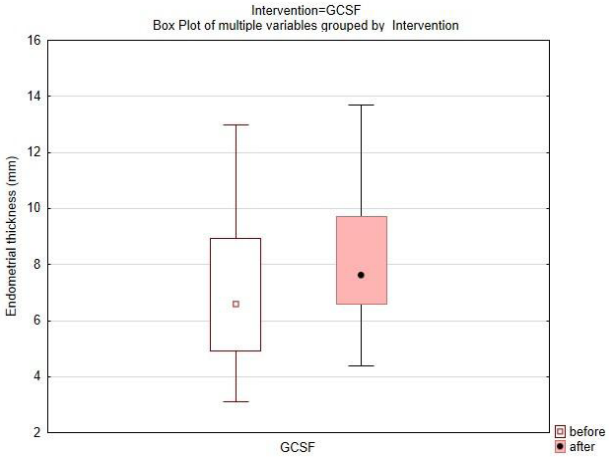


**Figure 11:** Boxplot of Endometrial thickness prior to and after G-CSF administration.

Paired t test

| Variable | Obs | Mean | Std. Err. | Std. Dev. | [95% Conf Interval] |
|---|---|---|---|---|---|
| xnum_e2 | 16 | 7.5 | 0.554602 | 2.218408 | 6.317894 - 8.682106 |
| xnum_e1 | 16 | 6.55625 | 0.5815188 | 2.326075 | 5.316772 - 7.795728 |
| diff | 16 | 0.94375 | 0.3044077 | 1.217631 | 0.2949203 - 1.59258 |

Mean (diff) = mean (xnum_e2 – xnum_e1) t = 3.1003
Ho: mean (diff) = 0 degrees of freedom = 15
Ha: mean (diff) < 0 Ha: mean (diff) !=0 Ha: mean (diff) > 0
Pr (T < t) = 0.9963 Pr ( |T| > |t| ) = 0.0073 Pr (T > t) = 0.0037

**Table 5**

The change in endometrial thickness for the PRP group ranged from 0.30 mm to 4.90 mm, whereas for the G-CSF group it ranged from 0.1 mm to 5.0 mm. A Mann Whitney u-test was done to compare the change in endometrial thickness for the respective interventions. This indicates that the change in the endometrial thickness for the PRP group (median: 1.05; Q1:0.63; Q3: 1.83) is not significantly greater than that of the change in endometrial thickness for the G-CSF group (median: 0.60; Q1: 0.53; Q3: 1.30) (U: 104.50; p-value= 0.077). Figure 12 indicates the comparison of the change in endometrial thickness for the two interventions.

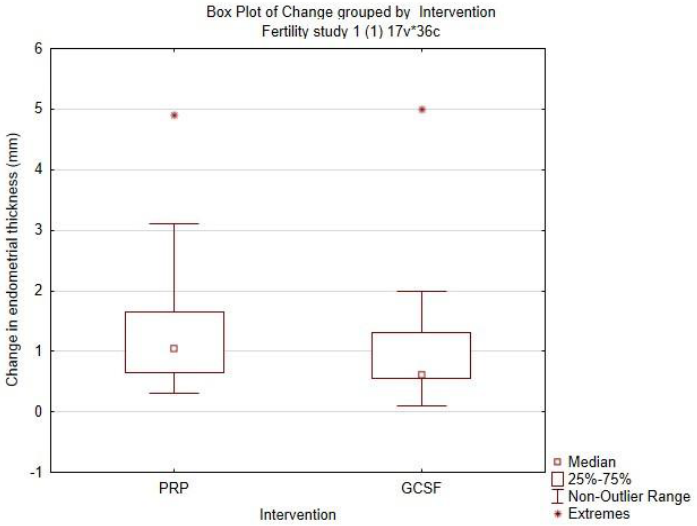


**Figure 12:** Boxplot of change in endometrial thickness in both the PRP and G-CSF groups.

## Pregnancy outcomes:

16 of the 36 women in the study had a positive pregnancy B-HCG test 10 days post ET. Of these, 9 received PRP and 7 received G-CSF as shown in Table 6 below. No statistically significant difference was noted between the two intervention groups (p-value = 0.604), in terms of pregnancy outcomes.

| PREGNANCY OUTCOME | Autologous PRP | | G-CSF | | Total Study Population | |
|---|---|---|---|---|---|---|
| | Freq | Per | Freq | Per | Freq | Per |
| Positive | 9 | 45.00 % | 7 | 43.75 % | 16 | 44.44 % |
| Negative | 11 | 55.00 % | 9 | 56.25 % | 20 | 55.56 % |

**Table 6:** Freq: Frequency; Per: Percentage.

The combined pregnancy rate in the intervention group was 44.44% with 16 out of 36 participants having established a positive pregnancy test. In the same period as our study, a total of 494 women underwent routine IVF therapy and embryo transfer without any adjunct therapies. Of these, 194 women had a positive pregnancy outcome with the overall pregnancy rate of 39.27%. This comparison is shown in Table 7 below. It is noted that the difference between the two groups was found not to be statistically significant (p-value= 0.352), which implies that patients receiving treatment had a pregnancy rate comparable to those undergoing routine IVF therapy. Importantly, this indicates that following therapy, patients with thin endometrium refractory to treatment achieve a pregnancy rate similar to patients who do not require such intervention.

Additionally, the pregnancy rate for the intervention group is substantially higher than that noted in the literature for patients with similar baseline endometrial thickness without any intervention. Reference pregnancy rates for frozen embryo transfers were noted to be 27.4%, 23.7% and 15.0% in patients with endometrial thickness of 7.0-7.9mm, 6.0-6.9mm and 5.0-5.9mm respectively [6]. It is important to note that in our study, no control group was included as it was deemed unsuitable by the authors to withhold potentially beneficial treatment from patients with thin endometrium.

| PREGNANCY OUTCOME | Study Population (G-CSF and PRP) | | IVF with no endometrial therapy | |
|---|---|---|---|---|
| | Freq | Per | Freq | Per |
| Positive | 16 | 44.44 % | 194 | 39.27 % |
| Negative | 20 | 55.55 % | 494 | 60.73 % |

**Table 7**

### Presence of endometrial fluid and pregnancy outcomes in each of the respective interventions:

2 patients in the PRP group and 4 in the G-CSF group had fluid in the endometrial cavity 48 hours prior to embryo transfer. A 50% positive pregnancy rate was observed in both groups; hence no difference was noted. This is shown in Table 8 below.

| In Participants with Fluid in The Endometrial Cavity | | | | |
|---|---|---|---|---|
| PREGNANCY OUTCOME | Autologous PRP | | G-CSF | |
| | Freq | Per | Freq | Per |
| Positive | 1 | 50.00 % | 2 | 50.00 % |
| Negative | 1 | 50.00 % | 2 | 50.00 % |

**Table 8**

## Discussion

The refractory thin preimplantation endometrium and fluid in the uterine cavity prior to ET remains a challenge for ART practitioners. Both have been independently associated with failed IVF-fresh and frozen ET cycles. Several causative factors have been postulated, including elevated or disrupted immune response, chronic endometritis, decreased endometrial gene regulation, abnormalities in the decidualization process, endometrial trauma and decreased endometrial blood flow [3]. Many of these factors are not reversable or curable.

Treatment modalities to date include “Endometrial Scratch”, fluid extraction, intravaginal or oral Sildenafil, Estrogen supplementation, electro- acupuncture, and the intrauterine administration of G-CSF and PRP. It is yet unclear which treatment options, singularly or in combination, will provide the optimum outcome.

Granulocyte colony stimulating factor (G-CSF), also known as colony stimulating factor 3 (CSF-3), is a polypeptide hematopoietic growth factor and cytokine that regulates the formation of polymorphonuclear neutrophils [10]. A study conducted by Fujii et al. [11] has shown that G-CSF may lead to improved reendothelialization and micro-vessel formation by elevating serum levels of the angiogenic cytokine Hepatocyte Growth Factor (HGF). It has also been suggested that G-CSF affects implantation through a poorly understood mechanism by acting on decidual macrophages [12]. The efficacy of intrauterine administration of G-CSF in expanding the thin endometrium in patients with thin endometrium was noted initially by Barad, et al. [13] and substantiated by others [14]. To the contrary, the meta-analysis by Li, et al. [15] found that “G-CSF was ineffective in increasing the endometrial thickness among infertile women undergoing IVF”, but that the “implantation rate, biochemical pregnancy rate, and clinical pregnancy rate were significantly higher”. However, a more recently updated meta- analysis by Xi et al., showed significant increase in endometrium thickness and clinical pregnancy rates following intrauterine administration of G-CSF [1].

Platelet rich plasma (PRP) is plasma derived from whole blood that has been enriched with platelets. Platelets contain several useful growth factors such as vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), platelet derived growth factor (PDGF), transforming growth factor (TGF) and other cytokines that stimulate cell proliferation [16]. It has been widely used in disciplines such as orthopedics and ophthalmology, mainly for its wound healing properties. A study by Chang, et al. in 2015 [17] showed that 5 women, all with poor endometrial response to standard IVF therapy, displayed successful endometrial expansion and pregnancy after the intrauterine administration of autologous PRP. Although the exact mechanism of action of the PRP has not yet been described, it has been proposed that the growth factors play a role in proliferation, apoptosis, inflammation, cell adhesion, chemotaxis, and immune responses during embryo implantation and further promotes the formation of decidual blood vessels, placental angiogenesis, and endometrial proliferation [18]. These properties potentially explain why women who receive this therapy generally show a significant improvement in their endometrial thickness and pregnancy outcomes as was observed in a randomized control trial by Eftekhar, et al. [2]. In contrast to the conclusions of these studies, a recent narrative review by Sharara et al. [19] which reviewed literature focusing on the use of PRP therapy in reproductive

medicine from databases including PubMed, MEDLINE and CINAHL Plus, found that only a few studies showed an increase in endometrial thickness, chemical and clinical pregnancy rates. However, Sharara et al. [19] note that lack of standardization of the PRP preparation and administration is a limitation and that larger randomized controlled clinical trials would be needed in future.

In terms of intrauterine therapy, it is unclear whether G-CSF or PRP is the better option. The cost of G-CSF is notably higher than that of PRP. Results from our study showed that both G-CSF and PRP caused significant expansion of the endometrium, but no statistically significant difference was noted between the two groups.

Our study further noted a significant increase in pregnancy rates in both treatment groups, surprisingly much higher than that reported previously in the literature. However, due to the small sample size, no definitive conclusion can be made in this regard. Concerning the effect on presence of fluid in the endometrial cavity, 50% of the study population in either group who had fluid in their endometrial cavities had a successful pregnancy. However, the patient numbers were extremely small (4 cases), precluding any definitive conclusions.

## Conclusion

Our study showed that the use of G-CSF and PRP intrauterine therapy for the refractory endometrium as adjunct to first line estradiol and Sildenafil use is effective in causing endometrial expansion. A statistically significant increase in endometrial thickness was observed in both the G-CSF and PRP groups. However, when comparing the change in endometrial thickness with respect to each modality of treatment (PRP vs G-CSF), no statistically significant difference was noted (p-value= 0.077). Of the 36 participants, 16 achieved a positive clinical pregnancy outcome as defined by a positive serum B-HCG test, yielding a positivity rate of 44.4%. Nine of the 16 participants (56.25%) received PRP, and seven of the 16 (43.75%) received G-CSF.

The inordinately high pregnancy rate in our intervention population, when benchmarked against literature expectations for similarly matched endometrial thickness patients not receiving any intervention deserves special mention. We believe this to be because in all the patients, intrauterine intervention was used as an adjunct to first line estradiol and sildenafil pre-treatment. In the case of the refractory thin endometrium, PRP appears to be equivalent to G-CSF in both endometrial expansion as well as pregnancy rates. It is also more affordable and accessible. Larger future studies are necessary to validate these findings.


## Acknowledgments

We acknowledge the following:

- Dr Admire Chikandiwa for his assistance with statistics and data analysis.
- University of Witwatersrand Faculty of Health Sciences Biostatistics Department for assistance with statistics and data analysis.


## References


1. Xie Y, Zhang T, Tian Z, et al. Efficacy of intrauterine perfusion of granulocyte colony-stimulating factor (G-CSF) for infertile women with thin endometrium: A systematic review and meta-analysis. Am J Reprod Immunol. 2017; 78.
2. Eftekhar M, Neghab N, Naghshineh E, et al. Can autologous platelet rich plasma expand endometrial thickness and improve pregnancy rate during frozen-thawed embryo transfer cycle? A randomized clinical trial. Taiwan J Obstet Gynecol. 2018; 57: 810-813.
3. Bashiri A, Halper KI, Orvieto R. Recurrent Implantation Failure-update overview on etiology, diagnosis, treatment and future directions. Reprod Biol Endocrinol. 2018; 16: 121.
4. Wang WJ, Zhang H, Chen ZQ, et al. Endometrial TGF-β, IL-10, IL-17 and autophagy are dysregulated in women with recurrent implantation failure with chronic endometritis. Reprod Biol Endocrinol. 2019; 17: 2.
5. Ranisavljevic N, Raad J, Anahory T, et al. Embryo transfer strategy and therapeutic options in infertile patients with thin endometrium: a systematic review. J Assist Reprod Genet. 2019; 36: 2217-2231.
6. Liu KE, Hartman M, Hartman A. Management of thin endometrium in assisted reproduction: a clinical practice guideline from the Canadian Fertility and Andrology Society. Reprod Biomed Online. 2019; 39: 49-62.
7. Liu KE, Hartman M, Hartman A, et al. The impact of a thin endometrial lining on fresh and frozen-thaw IVF outcomes: an analysis of over 40000 embryo transfers. Hum Reprod. 2018; 33: 1883-1888.
8. Peter Durairaj RR, Aberkane A, Polanski L, et al. Deregulation of the endometrial stromal cell secretome precedes embryo implantation failure. Mol Hum Reprod. 2017; 23: 478-487.
9. Vitagliano A, Saccardi C, Noventa M, et al. Effects of chronic endometritis therapy on *in vitro* fertilization outcome in women with repeated implantation failure: a systematic review and meta-analysis. Fertil Steril. 2018; 110: 103-112.e1.
10. Demetri GD, Griffin JD. Granulocyte colony-stimulating factor and its receptor. Blood. 1991; 78: 2791-2808.
11. Fujii K, Ishimaru F, Kozuka T, et al. Elevation of serum hepatocyte growth factor during granulocyte colony-stimulating factor-induced peripheral blood stem cell mobilization. Br J Haematol. 2004; 124: 190-194.
12. Barash A, Dekel N, Fieldust S, et al. Local injury to the endometrium doubles the incidence of successful pregnancies in patients undergoing *in vitro* fertilization. Fertil Steril. 2003; 79: 1317-1322.
13. Barad DH, Yu Y, Kushnir VA, et al. 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. Fertil Steril. 2014; 101: 710-715.

14. Mohamed T, Adam Y, Cassim MI. An evaluation of the use of G-CSF as an adjunct to IVF in women who have previously failed attempts at pregnancy with IVF. IJWHR. 2017; 7: 269-275.

15. Li J, Mo S, Chen Y. The effect of G-CSF on infertile women undergoing IVF treatment: A meta-analysis. Syst Biol Reprod Med. 2017; 63: 239-247.

16. Zadehmodarres S, Salehpour S, Saharkhiz N, et al. Treatment of thin endometrium with autologous platelet-rich plasma: a pilot study. JBRA Assist Reprod. 2017; 21: 54-56.

17. Chang Y, Li J, Li X, et al. Platelet-rich plasma administration has benefit for infertile women with thin endometrium in frozen blastocyst-stage embryos transfer program. Fertility and Sterility. 2017; 108: p.e77.

18. Farimani M, Poorolajal J, Rabiee S, et al. Successful pregnancy and live birth after intrauterine administration of autologous platelet-rich plasma in a woman with recurrent implantation failure: A case report. Int J Reprod Biomed. 2017; 15: 803-806.

19. Sharara FI, Lelea LL, Rahman S, et al. A narrative review of platelet-rich plasma (PRP) in reproductive medicine. J Assist Reprod Genet. 2021; 38: 1003-1012.