

## **Autologous intrauterine platelet rich plasma versus g-csf instillation for improvement of endometrial growth and vascularity in recurrent in vitro fertilization failure.**

### **Abstract:**

**Background**-in vitro fertilization[ivf] is an expensive,cumbersome yet most successful treatment for infertility.inspite of its success,at the most 40% of the procedure only result in good outcome.often at times which can be very frustrating and disappointing journey for the couple as well as the clinicians who despite of strict adherence to treatment protocols fail to produce result,it is mostly attributed to implantation failure,which is mostly due to poor receptivity of the endometrium.several treatments ranging from chinese acupuncture to hormones to endometrial injury all have been treat with not definitive treatment.autologous platelet rich plasma and g-csf have come up to be exciting new therapy in that aspect. Platelet-rich plasma (prp) is described to promote endometrial growth and receptivity while g-csf promotes the growth of stem cells and progenitor cells in neutrophilic granulocytes.this study provides an interesting prespective in comparison of these two drugs.

**Materials and methods**- It is a prospective ,cross-sectional, single blind study,conducted over a period of 6 months in 25 women who had failed to conceive after one or more embryo transfers with high-quality embryoswomen having a poor endometrial pattern, as defined by an endometrial thickness of less than 7mm despite conventional treatment with estradiol valerate (up to 12 mg/day), or suboptimal endometrial vascularity, defined as < zone 2 as determined by applebaum's criteria,success of which was measured in number of clinical pregnancies resulted.

**Results**- the mean pre platelet rich plasma endometrial thickness was 5 mm which significantly increased to 7.34 mm post platelet rich plasma,against post-g-csf endometrial thickness was 8.02 mm from 6.03mm pre infusion. Out of 10 cases who were infused with platelet rich plasma ,7 of them showed good vascularity (more than or equal to zone 3)out of 10 of those were given g-csf,5 of them showed good vascularity. Number of clinical pregnancies resulted after instillation of prp were 4 while 3 in case of g-csf group.

### **Conclusion-**

It can be concluded that although both platelet rich plasma and g-csf are equally effective in increasing endometrial thickness but endometrial vascularity is better improved with platelet rich plasma, clinical pregnancy rates were also better with platelet rich plasma but being a small sample study it was not significant stastically.

### **Introduction:**

In spite of more than 35 years of practice of in vitro fertilization, its success is still limited, mainly the interplay between mother's endometrium and embryo is responsible for its favourable outcome. Implantation is a complex and poorly understood process which involves various factors and requires a positive interaction between the embryo and the endometrium. One of the major variable factors for good implantation remains a good endometrial thickness and its vascularity. Several methods have been performed for successful embryo implantation and pregnancy like blastocyst transfer, pre-implantation genetic screening (pgs), assisted hatching, co-culture system, sequential transfer, hysteroscopy, endometrial scratching, salpingectomy for tubal disease, extra number of embryo transfer, natural cycle, oocyte donation, intra tubal embryo transfer, immune therapy and endometrial receptivity array (era) have been used but there is not any proven evidence in these treatments.(1)

Testing of adjuvant therapies such as use of growth hormone, androgens, and glucocorticoids to enhance oocyte number and quality, use of sildenafil, low-dose aspirin, heparin, corticosteroids, and granulocyte colony-stimulating factor (g-csf), endometrial injury, intrauterine injection of human chorionic gonadotropin [hcg], and intrauterine administration of autologous peripheral blood mononuclear cells to improve poor endometrial response; antioxidants, complementary and alternative medicine modalities, such as Chinese herbal medicine and acupuncture; and assisted hatching and preimplantation genetic screening to correct embryonic factors in properly conducted randomized controlled trials is rarely done so that potential benefits and risks are unlikely to be clearly presented to patients and clinicians.

Intrauterine infusion of platelet-rich plasma (prp) is described to promote endometrial growth and receptivity.(1) It is made from whole blood that contains many growth factors and cytokines such as fibroblast growth factor (fgf), platelet derived growth factor (pdgf), and vascular endothelial growth factor (vegf), transforming growth factor (tgf), insulin-like growth factors (igf-i,ii), connective tissue growth factor (ctgf) and interleukin 8 (il-8). Platelet rich plasma has been studied as a potential treatment for a variety of medical conditions, including nerve damage, ocular epithelial abnormalities, alopecia, heart muscle injury, osteoarthritis, and tendonitis. It has also been found to increase intrauterine endometrial growth and vascularity.

Endometrial doppler not only reflects on blood flow of endometrium but also indirectly reflects on angiogenesis required for implantation.

Preliminary studies demonstrated that g-csf stimulated neutrophilic granulocyte proliferation and differentiation, acted on macrophages of decidual cells, and finally affected the implantation. In addition, the known and reported immune effects of g-csf are recruitment of dendritic cells, promoting th-2 cytokine secretion, activating t regulatory cells, and also stimulation of various proangiogenic effects.(2) In theory, g-csf promotes the growth of stem cells and progenitor cells in neutrophilic granulocytes. Because of its proliferative effects on fibroblasts, g-csf might potentially increase endometrial thickness. However, more research has to be done in this area. Furthermore, it is uncertain how g-csf accomplishes fast endometrial architectural growth.(3)

The rationale behind this study is to assess the potential benefits of p-rp versus instillation of g-csf in intrauterine cavity, since both drugs have potential effect on endometrial growth and receptivity, yet no study exists so as to compare these two drugs. This study aims at assessing the more efficacious drug depending upon the rate of clinical pregnancy resulted.

## **Materials and methods**

**Study design :** this was a prospective, cross-sectional, single blind study done over 6 months which was conducted in Wardha Test Tube Baby Centre, Sawangi. 25 women who had failed to conceive after one or more embryo transfers with high-quality embryos and were chosen for frozen embryo transfer were evaluated for study eligibility. From March to July 2019, women having a poor endometrial pattern, as defined by an endometrial thickness of less than 7mm despite conventional treatment with estradiol valerate (up to 12 mg/day), or suboptimal endometrial vascularity, defined as < zone 2 as determined by Applebaum's criteria, were included in the study.

Participants gave their informed, valid, written consent.

Transvaginal ultrasonography measured endometrial thickness as the maximum distance between the echogenic surfaces of the myometrium and endometrium in the plane along the uterine body's central longitudinal axis. Endometrial Doppler zones were classified on the basis of blood flow reaching maximal endometrial depth-

If one divides the endometrial and periendometrial areas into the following four zones: (4)

Zone 1: a 2 mm thick area surrounding the hyperechoic outer layer of the endometrium.

Zone 2: the hyperechoic outer layer of the endometrium.

Zone 3: the hypoechoic inner layer of the endometrium.

Zone 4: the endometrial cavity.

The study also included women who had more than two cancelled cycles or recurrent implantation failure owing to a weak uterine lining. Any other known reason of implantation failure, such as low embryo quality, Asherman syndrome, or congenital uterine defects, was ruled out. Inclusion criteria

1. Women aged 22–45 years.
2. Women who had previous cycles cancelled because of thinly formed and poorly vascular endometrium in spite of treatment.
3. Implantation failure in previous cycle.
4. Normal shape and size of uterine cavity as confirmed by office hysteroscopy.

### **Exclusion criteria**

1. Women with history of any hematological and/or endocrine and/or renal disorder, history of any chronic medical disease.
2. Presence of uterine structural abnormality [congenital uterine anomalies/fibroid/polyp/Asherman's disease, septum, etc.]

Baseline tvs was done using voluson p 8 machine ic9-rs endovaginal probe with a frequency of 2.9mhz,operator bias was minimized by performing of transvaginal sonography by same sonologist **on day 2 of cycle.**administration estradiol valerate orally throughout the cycle, 1 mg of estradiol valerate was given on days 1 to 5, 2 mg on days 6 to 9, 6 mg on days 10 to 13, 2 mg on days 14 to 17, 4 mg on days 18 to 26, and 1 mg on days 27 and 28 **(5)**to all the participants, low-dose aspirin 150mg per day and vaginal sildenafil 25 mg per day were administered for endometrial preparation aswell. Endometrial thickness, pattern, vascularity and flow indices were assessed by tvs on day 10 in accordance with the slightly modified version of applebaum uterine scoring system was used. This uterine scoring system included all the following parameters: endometrial thickness, endometrial morphology, endometrial blood flow within zone 3, myometrial echogenecity, uterine artery pulsatility index (pi), end diastolic blood flow, and myometrial blood flow internal to the arcuate vessels seen on gray-scale examination.**(6)** if endometrial thickness was <7 mm, g-csf (300 mcg/1 ml) was instilled slowly into the uterine cavity using an intrauterine insemination (iui) canula under transabdominal ultrasound guidance following all aseptic precautions. Patients were asked to continue the estradiol preparation in the same dose as before. Endometrial thickness was assessed after 48 h and decision was made accordingly. If endometrial thickness was <7 mm, either a second infusion of g-csf was given or cycle was canceled, and if endometrial thickness was >7 mm, progesterone was started and embryo transfer was done on day 3.

in other group, after obtaining written informed consent, intrauterine infusion of autologous platelet rich plasma was instilled,in participants who failed to achieve endometrial thickness of 7 mm despite optimal dose of estradiol valerate for 10 days and poor vascularity.7.5 ml of blood was drawn from patient in a syringe containing anticoagulant. Platelet rich plasma was obtained using sequential centrifugation soft spin at 1200 rpm for 10 minutes,which separated the specimen in 3 layers,which then is given hard spin of 2000 rpm for 10 min,to obtain supernatant which is used for instillation in intrauterine cavity using an an intrauterine insemination iui canula under transabdominal ultrasound guidance following all aseptic precautions. Patients were asked to continue the estradiol preparation in the same dose as before.

repeat ultrasound was done 48 hrs later by the same observer and the endometrial thickness, pattern and vascularity was noted.second sitting of platelet rich plasma infusion was performed in a few patients who failed to show desired results.

In participants who had a good endometrium, frozen embryo transfer[with embryo of 2aa or 3aa quality] with a transfer of exactly one embryo, we excluded the number of embryos transferred as a variable.

There are two embryos. In addition, we kept embryo variability to a minimum.was performed (et of 7mm with moderate-to-excellent vascularity pattern). Appropriate luteal phase support was given, and 2 weeks later, the serum beta hcg level was assessed. Data was collected,tabulated and analysed.

## Statistical analysis

Data analysis software system, statistical package for social sciences (spss version 20) was used for statistical analysis. The mean sd of continuous data was used. For the continuous data, the paired t test and the wilcoxon rank sum test were used. A statistically significant p value of 0.05 was used.

## Results

Table 1- basic parameters of women in both the groups

Basic parameters	Platelet rich plasma group	G-csf group	P-value
Age	32.25±4.6	33.28±4.48	<0.17
Bmi	26.5±6.6	27.8±8	<0.72
S. Fsh	5.9±2.3	5.9±1.7	<1
S.lh	2.71± 3.4	2.89± 3.1	<0.22
Previous in vitro fertilization attempts	7.5±4.9	7.09±4.1	<0.51
Primary infertility	8	9	-
Secondary infertility	2	1	-

Table 1 depicts, the age group included in this study was from 22-45 years. Out of 25 patients 5 patients were excluded out of this study, 20 women were to be analysed who were further divided in two groups of 10 women each.

In platelet rich plasma group, out of 10 participants 3 participants were infused with single intrauterine platelet rich plasma and rest of them required 2 sittings of platelet rich plasma infusion..out of 10 participants with g-csf infusion, 5 required single sitting and rest 5 required double sitting.

Table 2-endometrial thickness

Study group	Pre infusion et [mean]	Post infusion et [mean]	Improvement in et	P value
Platelet rich plasma	5.96+/- 0.58mm	6.68+/- 0.84mm	0.72	<0.01
G-csf	6.03 +/- 0.53mm	6.85+/- 0.42mm	0.82	<0.01

As depicted in table 2, the mean pre platelet rich plasma endometrial thickness was 5 mm which significantly increased to 7.34 mm post platelet rich plasma, against post-g-csf endometrial thickness was 8.02 mm from 6.03mm pre infusion.

**Table 3- vascularity**

Study group	Poor vascularity	Good vascularity	Improvement in vascularity	P value
Platelet rich plasma	2.16 +/- 0.80	3.68 +/-0.23	1.52	<0.01
G-csf	2.19 +/-0.35	3.47 +/-0.02	1.28	<0.01

Table 3 depicted there was a significant increase in vascularity, seen by the number of vascular zones on color doppler, reaching the zones 3 and 4 of the endometrium on both the groups. Out of 10 cases who were infused with platelet rich plasma ,7 of them showed good vascularity (more than or equal to zone 3)out of 10 of those were given g-csf,5 of them showed good vascularity.

**Table 4 - clinical pregnancy rate**

Study group	Total number of cases	Number of biochemical pregnancies	Number of clinical pregnancies	% clinical pregnancies	P value
Platelet rich plasma	10	5	4	40%	<0.3
G-csf	10	4	3	30%	<0.22

Table 4 shows that the number of clinical pregnancies resulted after instillation of prp were 4 while 3 in case of g-csf group.although biochemical pregnancies were in 5 cases in prp group as to 4 in g-csf group.

**Fig 1. Before instillation of platelet rich plasma.**



**Fig 2. 48 hrs after instillation of platelet rich plasma, endometrial vascularity and thickness increased**



**Fig 3. endometrium before instillation of g-csf ,**



**Fig 4. endometrium after 48 hrs of instillation of g-csf, endometrial vascularity and thickness increased.**

## **Discussion**

platelet rich plasma can stimulate proliferation and regeneration with a large amount of growth factors and cytokines, including pdgf, tgf, vgef, egf, fibroblast growth factor (fgf), insulin-like growth factor i, ii (igf i, ii), interleukin 8 (il-8) and connective tissue growth factor (ctgf).(7)for the first time, chang studied the effectiveness of platelet rich plasma intrauterine infusion for endometrial development in women with thin endometrium. During the frozen embryo transfer cycle, platelet rich plasma was given into five women with insufficient endometrium who had a poor response to conventional treatment. All of them reported a positive reaction to therapy, and four of them reported a normal pregnancy.(8)

Vora et al concluded in patients with thin endometrium, gcsf instillation was found to be more efficient than intrauterine prp instillation in enhancing endometrial thickness, with a p-value of 0.0001. Chemical and clinical pregnancy rates were found to be comparable, with p values of 0.77 and 0.37, respectively,(9) indicating that they were not statistically significant. Despite the fact that patients who received injection gcsf had a slightly higher clinical pregnancy rate (44%) than those who received intrauterine prp, which had a rate of 28 percent, patients who received injection gcsf had a slightly higher clinical pregnancy rate (44%) than those who received

intrauterine prp, which was in contrast to present study where clinical pregnancy rate was higher in prp group at 40% while was 30% in who had g-csf.

In the implantation phase, endometrial receptivity and thickness are critical. According to our findings, gcsf intrauterine infusion on the day of ovarian puncture boosted clinical pregnancy rates. Our data demonstrated that intrauterine infusion considerably enhances the clinical pregnancy rate following art, similar to earlier studies that indicated g-csf injection may have a good influence on clinical pregnancy outcome after art. A prospective cohort study on four patients to examine the effect of gcsf on thin endometrium was one of the earliest investigations of gcsf. Gcsf is a novel therapy for thin endometrium, according to these cohort data.

## Conclusion:

According to this study, it can be concluded that although both platelet rich plasma and g-csf are equally effective in increasing endometrial thickness but endometrial vascularity is better improved with platelet rich plasma. Being autologous platelet rich plasma is free from transmission of infections and its preparation does not require much expenditure so it is cost effective too. Clinical pregnancy rates were also better with platelet rich plasma but being a small sample study it was not significant statically.

Drawbacks: as the cycles were fet cycles, patient were already on estrogen and low-dose aspirin and sildenafil, so we are not sure whether the small increment seen in endometrial thickness was an effect of g-csf or platelet rich plasma alone or it was due to a combined effect of all these preparations.

1. Nazari I, Salehpour S, Hoseini S, Zadehmodarres S, Ajori I. Effects of autologous platelet-rich plasma on implantation and pregnancy in repeated implantation failure: a pilot study. *Int J Reprod Biomed*. 2016 Oct;14(10):625–8.
2. Allahbadia. The unfinished story of granulocyte colony-stimulating factor in assisted reproductive technology [internet]. [cited 2022 Jan 5]. Available from: <http://www.ivflite.org/article.asp?issn=2348-2907;year=2014;volume=1;issue=3;spage=139;epage=145;aulast=allahbadia>
3. Gleicher N, Kim A, Michaeli T, Lee H-J, Shohat-Tal A, Lazzaroni E, et al. A pilot cohort study of granulocyte colony-stimulating factor in the treatment of unresponsive thin endometrium resistant to standard therapies. *Hum Reprod*. 2013 Jan 1;28(1):172–7.
4. Malhotra N, Tomar S, Malhotra J, Rao J, Malhotra N. Rational use of tvs/color and 3d in evaluating subfertile women. *Donald Sch J Ultrasound Obstet Gynecol*. 2011 Jul 1;5:273–87.
5. Devroey P, Pados G. Preparation of endometrium for egg donation. *Hum Reprod Update*. 1998 Nov 1;4(6):856–61.
6. Khan MS, Shaikh A, Ratnani R. Ultrasonography and Doppler study to predict uterine receptivity in infertile patients undergoing embryo transfer. *J Obstet Gynaecol India*. 2016 Oct;66(suppl 1):377–82.

7. Zadehmodarres s, salehpour s, saharhiz n, nazari l. Treatment of thin endometrium with autologous platelet-rich plasma: a pilot study. *Jbra assist reprod.* 2017 mar 9;21(1):54–6.
8. Chang y, li j, chen y, wei l, yang x, shi y, et al. Autologous platelet-rich plasma promotes endometrial growth and improves pregnancy outcome during in vitro fertilization. *Int j clin exp med.* 2015 jan 15;8(1):1286–90.
9. Vora av, nadkarni pk, garasia j, nadkarni a, singh pn, nadkarni vk. Intrauterine platelet rich plasma versus injection g-csf for treatment of thin endometrium in infertility. *Int j reprod contracept obstet gynecol.* 2019 sep 26;8(10):3931–8.