# *Original Research Article*

**Autologous Intrauterine Platelet Rich Plasma Versus G-CsfInstillation For Improvement Of Endometrial Growth And Vascularity In Recurrent In Vitro Fertilization Failure.**


## Abstract

Introduction:

This study was done to evaluate the effectiveness of PLATELET RICH PLASMA intrauterine instillation in infertile patients after recurrent IN VITRO FERTILIZATION failure.it was then compared with G-CSF and analysed in terms of effectiveness on endometrial thickness and vascularity when done with doppler study.

Material and methods

Intrauterine instillation of autologous platelet rich plasma was done in 10 patients against 10 patients who were given intrauterine G-CSF between age 22 and 38 years over 1 month with suboptimal endometrial growth and with prior cycle of In vitro fertilization failure.patients underwent serial ultrasounds along with doppler prior to implantation date. platelet rich plasma was prepared from autologous blood by centrifugation ,and 0.5 -1 ml of platelet rich plasma was infuses into the uterine cavity on the the 10th days of cycle. platelet rich plasma infusion and G-CSF infusion was done 1-2 times in each cycle seperately .embryos were transferred when the endometrial thickness was more than 7 mm with atleast zone 3 vascularity .

Results:

Successful endometrial growth and vascularity were observed as a primary parameters and clinical pregnancy was also noted as secondary outcome, in all patients with platelet rich plasma and G-CSF infusion.intrauterine platelet rich plasma infusion represent a new method for thin endometrium with poor responses.The mean pre platelet rich plasma , endometrial thickness (ET) was 5 mm which significantly increased to 7.34 mm post platelet rich plasma ,against


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post-G-CSF et which was 8.02 mm. There was significant increase in vascularity in platelet rich plasma group, reaching zones 3 to 4 of the endometrium on color doppler.clinical pregnancies were also higher in platelet rich plasma group,though statiscally not significant.

Conclusion:

This article reported that even though both platelet rich plasma and G-CSF were able to promote the endometrial growth of patients with thin endometrium but vascularity and clinical pregnancy outcome were better with platelet rich plasma, although it was not significant statistically.

## Introduction:

Inspite 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 it'sfavourableoutcome.Implantation is a complex and poorly understood process which involves various factors and requires a positive interaction between embryo and 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,sequentialtransfer,hysteroscopy,endometrialscratching,salpingectomy for tubal disease,extra number of embryo transfer transferred ,natural cycle,oocyte donation ,intra tubal et,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 growth hormone, androgens, and glucocorticoids to enhance oocyte number and quality; sildenafil, low-dose aspirin, heparin, corticosteroids, and granulocyte colony-stimulating factor (G-CSF), endometrial injury, intrauterine injection of human chorionic gonadotropin, 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.(2)

Intrauterine infusion of platelet-rich plasma (PRP) is described to promote endometrial growth and receptivity. Platelet rich plasma is prepared from fresh whole blood that contained several growth factors and cytokines including fibroblast growth factor (FGF), platelet derived growth factor (PDGF), vascular endothelial growth factor (VEGF), transforming growth factor (TGF), insulin-like growth factor i, ii (IGF-i, ii), connective tissue growth factor (CTGF) and interleukin

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8 (IL-8). Platelet rich plasma has been investigated as a therapeutic approach for several medical disorders including nerve injury, ocular epithelial defects, alopecia, cardiac muscle injury, osteoarthritis, and tendonitis.it has also been found to increase intrauterine endometrial growth and vascularity.(3)

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

# Materials and methods

Study Design :this was a prospective ,cross-sectional ,single blind study done over 6 month which was conducted in Wardha Test Tube Baby Centre,Sawangi.25 women who failed to conceive after 1 or more Embryo Transfer with high quality embryos and selected for frozen embryo transfer,were assessed foreligibility to enter the study .From march to july 2019, women with suboptimal endometrial pattern ,as indentified by Endometrial thickness <7mm despite standard done of estradiol valerate (upto 12 mg/day ),or suboptimal Endometrial vascularity ,defined as < zone 2 by Applebaum's criteria.

Participants gave their informed,valid,written consent.

Endometrial Thickness is measured by transvaginal ultrasound as the maximal distance between the echogenic interfaces ofthe myometrium and endometrium in the plane through central longitudinal axis of the uterine body.Endometrial doppler zones were classified on the basis of blood flow reaching maximal endometrial depthof Applebaum's grading.
Womenwithmore thantwo cancelled cycles or recurrent implantation failure due to poor endometrial lining were also included in the study.women with any other known cause of implantationfailure, such as poor embryo quality, Ashermannsyndrome, or congenital uterine anomalies, were excluded.

Inclusion criteria

1. Women aged 22–45 years.
2. Previous cycle cancellations because of thin and poorly vascular endometrium in spite of treatment.
3. Failure of previous cycle.
4. Normal uterine cavity as confirmed by office hysteroscopy.

Exclusion criteria

1. Presence of Systemic Diseases, Endocrine Disorders, Sickle Cell Disease, Chronic Neutropenia, And History of Malignancy and Renal Insufficiency.
2. Presence of Asherman's Syndrome, Fibroids, And Polyps on hysteroscopy.

Baseline TVS was done using Voluson E 8 machine on day 2 of cycle and estradiol valerate 8-12 mg per day, low-dose aspirin 150mg per day and vaginal Sildenafil 25 mg per day were administered for endometrial preparation. Endometrial thickness, pattern, vascularity and flow

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indices were assessed by TVS on day 10. If endometrial thickness was <7 mm, G-CSF (300 mcg/1 ml) was instilled slowly into the uterine cavity using an intrauterine insemination (IUI) catheter under usg guidance.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 performed 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 autologousPlatelet Rich Plasmawasdone , who failed to achieve endometrial thickness of 7mm 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 IUI canula under ultrasound guidance following all aseptic precautions.patients were asked to continue the estradiol preparation in the same dose as before.
Repeat ultrasound was done 48hrs later by the same observer and the ET, pattern and vascularity was noted.Second sitting of Platelet Rich Plasmainfusion was performed in a few patients who failed to show desired results.

Frozen embryo transfer was done in the patients who achieved a satisfactory endometrium (ET of 7mm with Moderate-to-excellent vascularity pattern). Appropriate luteal phase support was provided and serum beta HCG Level was measured 2 weeks later.

Data was collected,tabulated and analysed.

## Statistical Analysis

Statistical analysis was done using data analysis software system, statistical package for social sciences (SPSS Version 20). Continuous data were expressed as mean ± sd. Paired t test and wilcoxon rank sum test were Applied for the continuous data. P value of 0.05 was considered statistically significant.

# Results

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 analysedwho were further divided in two groups of 10 women each.
In Platelet rich Plasmagroup ,out of 10 participant 3 participants were infused with single intrauterine platelet rich plasma and rest of them required 2 sittings ofPlatelet Rich Plasmainfusion..Out of 10 participants with G-CSF infusion,5 required single sitting and rest 5 required double sitting.
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.
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 .

# Tables-

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Table 1-Basic Parameters Of Women In Both The Groups

| Study Group | Pre Infusion ET [Mean] | Post Infusion ET [Mean] | Improvement In ET | P Value |
|---|---|---|---|---|

| 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 | - |

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Table 2-Endometrial Thickness

| | | | | |
|---|---|---|---|---|
| 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 |

## 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 |

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## 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 |

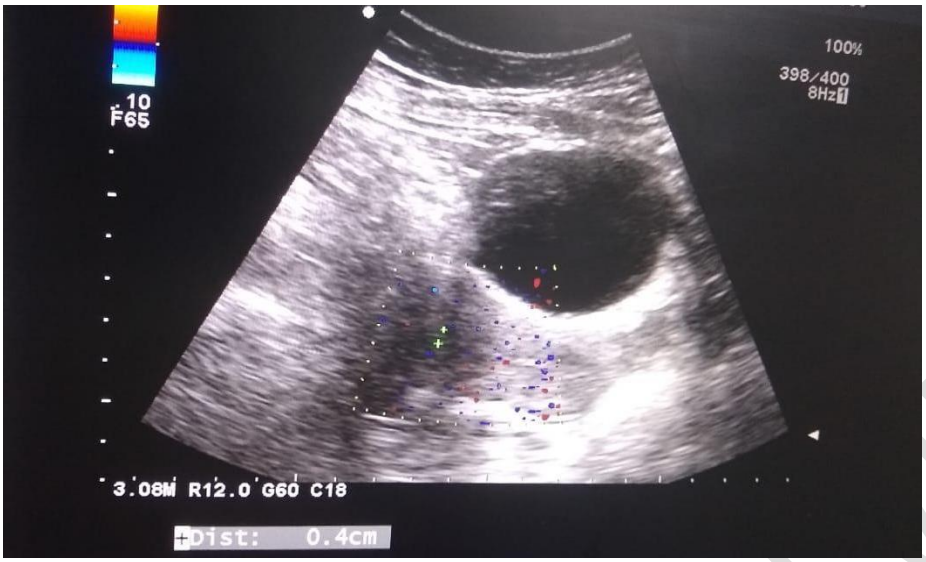


**Fig 1. Before instillation of Platelet Rich Plasma**.

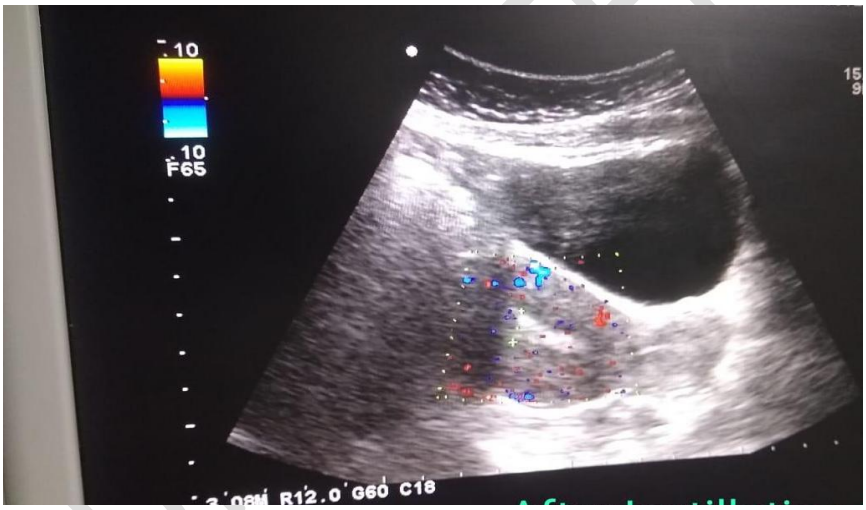


**Fig 2. After instillation of PLATELET RICH PLASMA,Endometrial vascularity and thickness increased**

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**Fig 3.Endometrium before instillation of G-CSF ,**


**Fig 4.Endometrium after 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 (IGFi, ii), interleukin 8 (IL-8) and connective tissue growth factor (CTGF). (3)

For the first time, Chang reported the efficacy of intrauterine infusion of Platelet Rich Plasma for endometrial growth in women with thin endometrium. In that trial, Platelet Rich Plasmawas infused in 5 women with inadequate endometrium who had poor response to conventional therapy during the frozen embryo transfercycle.The proper response to treatment was reported in all of them, and normal pregnancy was reported in 4 women (4).

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The endometrial receptivity and thickness play essential role in implantation phase (5,6). Based on our study, clinical pregnancy rate was improved by GCSF intrauterine infusion on ovarian puncture day. Similar to previous studies that indicated G-CSF administration may have beneficial effect on clinical pregnancy outcome after ART our findings showed that intrauterine infusion significantly improves the clinical pregnancy rate (7). One of the first studies of GCSF was a prospective cohort study on 4 patients to evaluate the effect of GCSF on thin endometrium. These cohort findings represented GCSF is a new treatment of thin endometrium (8).

## Conclusion:

According to this study, it can be concluded that although both platelet rich plasmaand G-CSF are equally effective in increasing endometrial thickness but endometrial vascularity is better inproved with platelet rich plasma. Being autologous platelet rich plasma is free from transmission of infections and it's prepaprationdonot require much expenditure so it is cost effective too. Clinical pregnancy rates were also better with platelet rich plasmabut being a small sample study it was not significant stastically..

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

NOTE:

The study highlights the efficacy of "HERBAL MEDIICINE which is an ancient tradition, used in some parts of India. This ancient concept should be carefully evaluated in the light of modern medical science and can be utilized partially if found suitable.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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