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Original Article

Obstetrics and Gynaecology Section

# Comparison of Platelet Rich Plasma Therapy, Granulocyte Colony Stimulating Factor, and Estradiol Valerate on Endometrial Thickness in Patients with Infertility: A Prospective Interventional Study

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## ABSTRACT

**Introduction:** According to World Health Organisation (WHO) infertility is "a disease of the reproductive system defined by the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse".

**Aim:** To compare the effect of Platelet Rich Plasma (PRP) therapy, Granulocyte Colony Stimulating Factor (GCSF) and estradiol valerate on endometrial thickness in infertile patient with thin endometrium.

**Materials and Methods:** The present prospective interventional, single-blind study was conducted on patients attending the outpatient clinics with complaints of infertility in the Department of Obstetrics and Gynaecology, GSVM Medical College, Kanpur, India, from January 2019 to September 2020. A total of 75 patients attending the outpatient clinics with infertility were included. The patients were divided randomly into three groups of 25 each. Patients in the group 1 were given PRP 0.1 mL infusion following ovulation induction with letrozole. Patients in group 2 were given GCSF infusion following ovulation induction with letrozole and patients in group 3 were given estradiol valerate following ovulation induction with letrozole. They were then instructed to come on day 13 when Transvaginal Scan (TVS) was done to assess the thickness and character of endometrium. Kruskal Wallis Test and paired t-test were used.

**Results:** Mean age of the patients was 28.1±4.36 years. The change (increase) in endometrial thickness is significant in the PRP group compared to the GCSF group and highly significant compared to the estradiol valerate group (4.19±2.06 vs 2.78±1.72 vs 1.94±1.69). However, the increase in endometrial thickness is comparable among GCSF and EV groups. The pregnancy rate was higher in PRP compared to GCSF and EV (32% vs 8% vs 20%).

**Conclusion:** It was found that the local administration of PRP is significantly effective in increasing endometrial thickness thus improving the possibility of pregnancy in patient with refractory endometrial thickness (<6mm).


**Keywords:** Implantation, Pregnancy, Unexplained infertility

## INTRODUCTION

According to WHO infertility is "a disease of the reproductive system defined by the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse." One in every four couples in developing countries had been found to be affected by infertility [1]. Approximately, 8% of currently married women suffered from infertility in India and most of them were secondary infertile (5.8%) [2].

There are multiple factors responsible for infertility and at times many are unexplained. Even after extensive diagnostic workup, aetiology in 10-25% of the couples remains unexplained and this has highlighted the importance of implantation and endometrial receptivity which depends on the following morphological factors like the thickness of the endometrium, echogenic pattern of the endometrium, endometrial and sub endometrial blood flow [3]. PRP is a plasma fraction of autologous blood with the concentration of platelets 4-5 times above normal [4]. With the functions of platelets, PRP is now a days widely applied in clinical scenarios, even in mucous tissues such as eyes [5] and mouth to improve tissue regeneration.

GCSF is a glycoprotein synthesised by mononuclear cells (e.g., macrophages), fibroblasts, endometrial cells, and Natural Killer (NK) cells. GCSF exerts its influence at various levels of the implantation process, making it an attractive diagnostic and therapeutic tool [6].

The need for oestrogen in the follicular phase of the cycle is beyond question, as oestrogen helps endometrial proliferation by causing spinal artery contraction and reducing oxygen tension in the functional layer, which facilitates embryo implantation [7]. This study aimed to compare the effectiveness of platelet-rich plasma therapy on endometrial thickness and pregnancy rate and its comparison with GCSF and estradiol valerate.

## MATERIALS AND METHODS

A prospective interventional, single-blind study was conducted on patients attending outpatient clinics with complaints of infertility in the Department of Obstetrics and Gynaecology, GSVM Medical College, Kanpur, India, from January 2019 to December 2020. All procedure performed in this study were in accordance with the ethical committee, GSVM Medical College. Ref No-17/E.C/Acad./12-04-2019. All 75 patients attending the outpatient clinics with infertility after fulfilling the inclusions and exclusions criteria and after proper written and informed consent were included.

**Inclusion criteria:**

- Patient willing to participate in the study;
- Patients with primary or secondary infertility in reproductive age group showing bilateral free spillage on hysterosalpingography or after diagnostic laparoscopy and hysteroscopy;
- Mature ovulatory cycle either spontaneously or by ovulation induction;

- Normal uterus and hormonal profile;
- Patient with persistent thin endometrium of <7 mm on >1cycle in previous ovulation induction cycle.

**Exclusion criteria:**

- Patient who does not give consent for the study;
- Age <18-year-old or >45-year-old
- Diagnosis of cancer or any significant co-morbidity or psychiatric disorder that would compromise patient safety or compliance.
- Presence of any organic lesions of uterus, tubes or ovaries such as endometrial polyp, ovarian cyst, leiomyomas.
- Endocrine abnormalities such as hyperprolactinaemia or abnormal thyroid functions.
- Male factor infertility
- Those with Body Mass Index (BMI) >30 kg/mm$^2$, repeated (>3) abortion, congenital or acquired uterine anomaly.

## Study Procedure

All couples with infertility were assessed initially by full history and then by general clinical and gynaecological examination. Basic semen analysis was done to rule out male factor infertility. Basal hormonal profile and hysterosalpingogram was done. TVS for imaging uterus and adnexa for any pathology, baseline TVS was done to assess the endometrial thickness and echogenicity on day 12. Patient were selected randomly and then allotted the three groups again on random basis by generating random numbers on computer.

**Group 1:** A total of 25 patients in group 1 were given PRP infusion following ovulation induction with letrozole. PRP was prepared from autologous blood using a two-step centrifuge process. On the 9$^{th}$ or 10$^{th}$ day of the menstrual cycle, 17.5 mL of peripheral venous blood was drawn in the syringe that contains 2.5 mL of Acid Citrate Anticoagulant solution (ACD-A) and was centrifuged immediately at 1200 rpm for 12 min to separate the red blood cells. The plasma was centrifuged again at 3300 rpm for seven minutes to obtain the PRP. Then, 1 mL of PRP was infused into the uterine cavity with the Intrauterine Insemination (IUI) catheter and then evaluated by TVS on day 13 to assess the endometrial thickness.

**Group 2:** A total of 25 patients in group 2 were given GCSF infusion following ovulation induction with letrozole. A 300 microgram GCSF was given with IUI catheter on day 13 to assess the endometrial thickness.

**Group 3:** A total of 25 patients in group 3 were given EV following ovulation induction with letrozole. Estradiol valerate was given to the patient in a dose of 2 mg six hourly from day 8 to day 13 then evaluated by TVS on day 13 to assess the endometrial thickness.

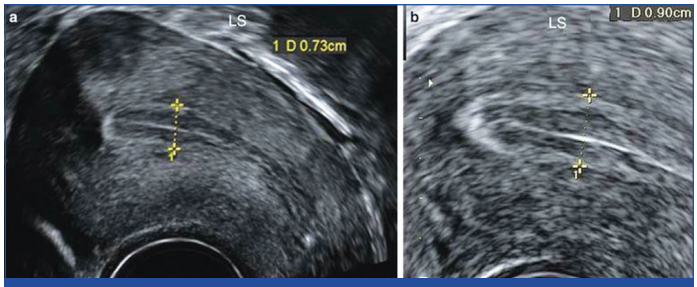
They were instructed to come on day 13 when TVS was done to assess the thickness and character of endometrium. Endometrial thickness was defined as the minimum distance between the echogenic interfaces of the myometrium and endometrium measured in the plane through the central longitudinal axis of the uterine body [Table/Fig-1]. Endometrial Thickness was measured thrice by a single observer and the mean value was taken. The results were also compared with pretreatment values in all three study groups.

## STATISTICAL ANALYSIS

Data analysis was done using IBM Statistical Package for Social Sciences (SPSS) Statistics version 22.0. It was found that the data was not normally distributed, so KRUSKAL WALLIS TEST and paired t-test were used.

## RESULTS

The study participants mean age was 28.1±4.36 years. Maximum patient belong to urban habitat. Most of the patients were of normal BMI as obese patients were excluded in the present study [Table/Fig-2].




**[Table/Fig-1]:** Measurement of endometrial thickness.

| S. No. | Clinicosocial profile | | PRP n (%) | GCSF n (%) | EV n (%) |
|---|---|---|---|---|---|
| 1 | Age (mean age 28.1±4.36 years) | 20-24 | 3 (12) | 5 (20) | 2 (8) |
| | | 25-29 | 14 (56) | 12 (48) | 15 (60) |
| | | >30 | 8 (32) | 8 (32) | 8 (32) |
| 2. | Residential status | Rural | 9 (36) | 10 (40) | 11 (44) |
| | | Urban | 16 (64) | 15 (60) | 14 (56) |
| 3. | Education | Literate | 10 (40) | 9 (36) | 10 (40) |
| | | Illiterate | 15 (60) | 16 (64) | 15 (60) |
| 4. | Religion | Hindu | 14 (56) | 16 (64) | 19 (76) |
| | | Muslim | 8 (32) | 7 (28) | 4 (16) |
| | | Others | 3 (12) | 2 (8) | 2 (8) |
| 5. | BMI | <18.5 | 0 | 2 (8) | 0 |
| | | 18.5-24.9 | 17 (68) | 17 (68) | 16 |
| | | 25-29.9 | 8 (32) | 6 (24) | 9 (36) |
| | | >30 | 0 | 0 | 0 |
| 6. | Type of infertility | Primary | 15 (60) | 14 (56) | 18 (72) |
| | | Secondary | 10 (40) | 11 (44) | 7 (28) |
| 7. | Duration of infertility (years) | <4 | 12 (48) | 10 (40) | 12 (48) |
| | | 4-5.9 | 7 (28) | 5 (20) | 7 (28) |
| | | 6-7.9 | 5 (20) | 7 (28) | 3 (12) |
| | | 8 and above | 1 (4) | 3 (12) | 3 (12) |

**[Table/Fig-2]:** Clinicosocial profile of patients.

[Table/Fig-3] shows that the increase in endometrial thickness after treatment by PRP is significantly higher. The mean increase was 4.19±2.06. The increase was highly significant (t=10.17, p=0.0001).

The change (increase) in endometrial thickness was significant in the PRP group compared to the GCSF group and highly significant compared to the estradiol valerate group. However, the increase in endometrial thickness was comparable among GCSF and EV groups [Table/Fig-4]. The pregnancy rate was higher in PRP as compared to GCSF and EV (32% vs 8% vs 20%) [Table/Fig-5].

## DISCUSSION

It was seen that in the present study maximum number of infertile patients were between age group 25-29 years (41 out of 75). The mean age was 28.1±4.36 years. Eftekhar M et al., conducted a similar study in which the mean age group was 31.98±2.26 in the PRP group and 32.40±2.63 in the control group [8]. The majority of patients 47 (62.7%) out of 75 had primary infertility while 28 (37.3%) patients had secondary infertility. The present study is comparable to the result of Eftekhar M et al., (primary infertility 72.5% and secondary infertility 27.5%) and to Kunicki M et al., (primary in fertility 64.86% and secondary infertility 42.86%) [8,9].

In the present study, the maximum number of patients (34) were having duration of infertility is <4 years and the mean duration of infertility 4.4±2.1 years. Similar findings were observed by Kim H

| Changes treatment ET after-ET before | Mean change after treatment | | | | | Paired 't' | p-value |
|---|---|---|---|---|---|---|---|
| | Mean | S.D. | Std. Error mean | 95% CI of the difference | | | |
| | | | | Lower | Upper | | |
| Platelet Rich Plasma (PRP) | 4.19 | 2.06 | 0.41 | 3.34 | 5.04 | 10.17 | 0.0001 |
| Granulocyte Colony Stimulating Factor (GCSF) | 2.78 | 1.72 | 0.35 | 2.06 | 3.48 | 8.09 | <0.001 |
| Estradiol Valerate (EV) | 1.94 | 1.69 | 0.34 | 1.24 | 2.64 | 5.78 | <0.001 |

**[Table/Fig-3]:** Change in endometrial thickness.

| Treatment pairs | Test statistics | p-value* |
|---|---|---|
| PRP vs GCSF | 13.66 | 0.027 |
| PRP vs EV | 22.86 | <0.001 |
| GCSF vs EV | 9.16 | 0.137 |

**[Table/Fig-4]:** Comparison of treatment groups.
*Kruskal Wallis Test

| S No. | Intervention | No. of pregnancy | Percentage |
|---|---|---|---|
| 1 | PRP | 8 (25) | 32% |
| 2 | GCSF | 2 (25) | 8% |
| 3 | EV | 5 (25) | 20% |
| | Total | 15 (75) | 20% |

**[Table/Fig-5]:** Pregnancy rate.

et al., the mean duration of infertility was 5.7±2.6 years [10]. Sipahi M et al., found the mean duration of infertility was 2.33±1.2 and 1.58±0.7 in the PRP group and control group [11]. In contrast to this, Aghajanzadeh F et al., found duration of infertility of 11.3±1.58 years in the study group [12].

Mean Endometrial Thickness (ET) was significantly increased in all three groups after treatment. Mean change after treatment with PRP, GCSF, and EV are 4.19±2.06, 2.78±1.71 and 1.94±1.68. Similar observations were made by Kunicki M et al., and Tendulwadkar S et al., [9,13]. The present study is also supported by the study of Chang Y et al., in which statistically significant increase in ET was found in PRP group in comparison to control group (7.65±0.22mm) [14]. Deo A et al., concluded that both PRP and GCSF are equally effective in increasing endometrial thickness [15].

On comparing GCSF with EV we did not find significant difference between their effects on ET. This is in accordance with Kalem Z et al., with no difference was observed between the study and control groups regarding endometrial thickness (p=0.965) [16]. But in contrast, Mishra VV et al., found a significant increase in ET after administration of GCSF in 19 out of 35 patients [17].

The pregnancy rate in the current study was higher in the PRP group as compared to GCSF and EV groups (32% vs 8% vs 20%). This was similar to the study conducted by Mehrafza M et al., in which the clinical pregnancy rate was significantly higher in PRP group than GCSF group (40.3% versus 21.4%, p=0.025) [18].

### Limitation(s)

Since the sample size is small to show a statistically significant result on ET improvement, and pregnancy rates. Further studies on the molecular basis of PRP treatment and well-designed Randomised Control Trial (RCT) are necessary to reveal the exact mechanism and to obtain more solid evidence on the beneficial effect of PRP on the endometrium of various pathophysiology.

## CONCLUSION(S)

It was found that the local administration of PRP is significantly effective in increasing endometrial thickness thus improving the possibility of pregnancy in patient with refractory endometrial thickness (<6 mm). This procedure, if used routinely in practice, would reduce not just physical but also the financial and psychological burden faced by such patients.

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