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Original Research Article

Role of Autologous Platelet-Rich Plasma in Thin Endometrium in *In* Vitro Fertilization

Vineet Mishra[1], Zainab Raja[2], Rohina Aggarwal[3], Sumesh Choudhary[4], Kunnur Shah[5], Meet Patel[6]

[1]Director and Head, Department of Obstetrics & Gynecology, Institute of Kidney Diseases and Research Center, Civil Hospital Campus, Ahmedabad

[2]Assistant Professor, Department of Obstetrics & Gynecology, Institute of Kidney Diseases and Research Center, Civil Hospital Campus, Ahmedabad

[3]Professor, Department of Obstetrics & Gynecology, Institute of Kidney Diseases and Research Center, Civil Hospital Campus, Ahmedabad

[4]Professor, Department of Obstetrics & Gynecology, Institute of Kidney Diseases and Research Center, Civil Hospital Campus, Ahmedabad

[5]Associate Professor, Department of Obstetrics & Gynecology, Institute of Kidney Diseases and Research Center, Civil Hospital Campus, Ahmedabad

[6]Senior Resident, Department of Obstetrics & Gynecology, Institute of Kidney Diseases and Research Center, Civil Hospital Campus, Ahmedabad

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Corresponding author: Dr. Zainab Raja

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Abstract

Background and Aim: Patients with infertility still face unresolved issues related to thin or damaged endometrium. Endometrial thickness (EMT) is empirically preferred to be >7 mm among doctors, and refractory thin endometrium, which does not respond to conventional medical therapy, may be the cause of recurrent implantation failure (RIF). In the present study, we conducted Platelet-Rich Plasma (PRP) treatment and investigated its effect on the refractory thin endometrium.

Material and Methods: 120 patients were the participants of a prospective study that ran from March 2022 to March 2023 at the Tertiary Care Teaching Hospital in India's Department of Gynecology. After more than two cycles of prior medical therapy for enhancing the EMT, the primary inclusion criteria were EMT of less than 7 mm. From menstrual cycle day 10 of their frozen-thawed embryo transfer (FET) cycle onward, the participants received treatment with intrauterine infusions of autologous PRP twice or three times. ET was carried out three days after the final autologous PRP infusion.

Results: The final oestrogen priming day or the previous-cycle hCG injection had a mean EMT of 5.4 mm. Thirty percent of the patients (n = 36) had the gestational sac verified. (n = 24) The live birth rate was 20%. All of the current pregnancies ended in live births free of obstetric issues. After receiving PRP therapy, the mean EMT was 6.2 mm. The EMT had an average increase of 0.7 mm. This distinction, nevertheless, was not statistically significant. The results of the treatment cycle were contrasted with those of each patient's most recent ET cycle, which served as the control cycle. In the treatment cycle, the rates for clinical pregnancy, live births, and implantation were 12.6, 30, and 20%, respectively.

Conclusion: An innovative method for helping infertile women with thin, resistant endometrium conceive more often is autologous PRP. PRP is free of adverse effects because it is made from the patient's own blood. Also, because it is readily available, affordable, and administered locally, it can be a blessing for patients who are infertile.

Keywords: Endometrial Thickness, Frozen-thawed embryo transfer, Gestational Sac, Platelet-Rich Plasma

Introduction

One of the key elements in implantation and pregnancy is the endometrium. Growing endometrial thickness is correlated with an increased pregnancy rate. The minimum endometrial thickness for embryo transfer has been found to be 7 mm in a number of investigations. [1,2] In frozen-thawed embryo transfer (FET) cycles, endometrial preparation is carried out using a variety of techniques, and the most efficient approach is not universally agreed upon. Despite routine therapy, some FET cycles are stopped because of thin endometrium, and there is no clear regimen for this situation. Prolonged oestrogen therapy and adjuvant therapy, including intrauterine perfusion with granulocytecolony stimulating factor (G-CSF), low dosage aspirin, vaginal Sildenafil, and pentoxifylline, have been utilised for thin endometrium, however there isn't any conclusive evidence to support these approaches. [3-9]

Recurrent implantation failure (RIF) is defined as the absence of the gestational sac on ultrasound at 5 weeks or more after a frozen embryo transfer (FET) following 3 FET with high-quality embryos or after the transfer of 10 or more embryos in multiple transfers, according to the European Society of Human Reproduction and Embryology (EHRE) consortium. [10,11] Repeated implantation failure is a significant problem in reproductive medicine, and despite many advancements, there is still no accepted solution. Several methods, including oestrogen, low-dose aspirin, heparin, vaginal sildenafil, pentoxifylline, and intrauterine perfusion of granulocyte-colony stimulating factor (G-CSF), have been widely utilised to boost the ET if not to the desired level. [12,13] Unfortunately, it was discovered that these techniques were not always very effective, particularly when dealing with a thin refractory endometrium. When repeated implantation attempts owing to a thin endometrium fail, platelet-rich plasma (PRP) may be useful in encouraging endometrial development, boosting ET, and enhancing endometrial vascularity, as well as improving pregnancy outcomes.

In 2015, a study using PRP to treat human thin endometrium in vivo was released. [14] PRP is an effective treatment for thin endometrium, according to four investigations that came after. [15-18] They claimed that autologous PRP encourages endometrial development and enhances the success of conception. Nevertheless, there were only a few patients, and they didn't give enough details about the kind or quantity of PRP they utilised. It is well established that the platelet concentration and cell composition of PRP might affect its efficacy.[19] VEGF, transforming growth factor (TGF), platelet-derived growth factor (PDGF), and epidermal growth factor (EGF) are just a few of the cytokines and growth factors found in platelet-rich plasma, which supports cellular migration, proliferation, and growth. Platelet-rich plasma is a fraction of

plasma made from autologous blood that is highly enriched with platelets.[20] Platelets naturally have the ability to promote healing by secreting a variety of growth factors, hence platelet-rich plasma has been employed in a variety of sectors like dermatology, dentistry, orthopaedics, and sports medicine.[21] In the current study, we treated patients with PRP and looked at how it affected the refractory thin endometrium.

Material and Methods

120 patients were the subjects of a prospective study that conducted from March 2022 to March 2023 at the Tertiary Care Teaching Hospital in India's Department of Gynecology. Patients who had previously experienced FET cycle cancellations due to insufficient endometrial development (less than 7 mm) while receiving conventional treatments were enrolled in the study.

The following were the inclusion requirements: The following criteria must be met: (a) age between 20 and 45 years old at the time of enrollment; (b) endometrial thickness (EMT) of less than 7 mm on the day of hCG administration in fresh ET cycles or on the last day of oestrogen priming in frozen ET cycles in all of the preceding cycles; (c) two or more unsuccessful IVF cycles; (d) more than two cycles of prior therapy for increasing the EMT, such as hysteroscopic adhesio The following were the exclusion requirements. (a) hematologic disorders, hemoglobin level of 9.0 g/dL or platelet count of 100,000/μL, (b) auto-immune disease, (c) chromosomal abnormality in the patient or spouse, (d) peripheral NK cell proportion of ≥12%, (e) body mass index (BMI) of ≥30 kg/m2 and (f) uncontrolled endocrine or other medical conditions, such as prolactinemia or thyroid diseases.

Autologous PRP Preparation

Using 30 mL syringes coated with 2 cc of acid citrate A, an anticoagulant solution, 18 mL of venous blood was taken from the patients on each day that PRP was administered. The blood samples were then placed into an aseptic PRP centrifuge kit and spun at 1017 G for 3 min. After gathering the buffy coat and the plasma right above it, 0.7–1.0 mL of PRP was created and pumped into the uterine cavity. According to the manufacturer's data, the PRP's platelet content varied between 717 and 1565 103/L and its WBC concentration between 24,000 and 37,000/L.

If it hadn't been done before, a hysteroscopic examination was done before the cycle. During the estrogen-primed FET cycle, intrauterine autologous PRP administration was carried out. To prepare the endometrium, the patients began taking a daily dose of 4-6 mg of estradiol valerate on menstrual cycle day (MCD) 2. Until the EMT reached 7 mm, the first autologous PRP infusion was carried out on Day 10 and was subsequently repeated three days later. Within 1 hour of finishing PRP preparation, PRP was injected via an ET catheter into the uterine cavity. The ET catheter was attached to the syringe containing the PRP, and the PRP was then injected.

The leftover PRP was then injected using the syringe that had been filled with air. If it hadn't been done before, a hysteroscopic examination was done before the cycle. During the estrogen-primed FET cycle, intrauterine autologous PRP administration was carried out. To prepare the endometrium, the patients began taking a daily dose of 4-6 mg of estradiol valerate on menstrual cycle day (MCD) 2. Until the EMT reached 7 mm, the first autologous PRP infusion was carried out on Day 10 and was subsequently repeated three days later. Within 1 hour of finishing PRP preparation, PRP was injected via an ET catheter into the uterine cavity. The ET catheter was attached to the syringe containing the PRP, and the PRP was then injected. The leftover PRP was then injected

using the syringe that had been filled with air. Individuals who had a positive hCG test got another ultrasound 2 weeks later to verify their clinical pregnancies. An intrauterine gestational sac was used to determine clinical pregnancy. Up until nine weeks of pregnancy, the luteal phase support was maintained. A timely chart review was used to monitor the expectant patients' obstetric progress.

The most recent ET cycles' factors and the treatment cycle's variables were compared. The continued pregnancy rate and LBR were the main results. The implantation rate, clinical pregnancy rate, and EMT increase in comparison to the previous cycle were the secondary endpoints.

Statistical analysis

The recorded data was compiled and entered in a spreadsheet computer program (Microsoft Excel 2007) and then exported to data editor page of SPSS version 15 (SPSS Inc., Chicago, Illinois, USA). For all tests, confidence level and level of significance were set at 95% and 5% respectively.

Results

120 of the 125 women that were recruited underwent ET. The patients were 35.7 years old on average. Infertility affected 120 women on average for 5.5 years, and 1.3 dilatation and evacuation procedures were performed on average. There were 2.5 failed IVF cycles on average. The final oestrogen priming day or the previous-cycle hCG injection had a mean EMT of 5.4 mm. During hysteroscopy, 16 of them were identified as having endometrial sclerosis or adhesion; the underlying causes were radiation therapy for the treatment of pelvic TB in one patient and colon cancer in another. (Table 1)

Two or three embryos were transplanted into each patient. A grade I or grade II cleavage stage embryo with six or more cells and a blastocyst score of 3BB or higher was considered to be a good-quality embryo. The morula was regarded as an excellent embryo. 18 individuals had solely poor-grade cleavage embryos, while 102 patients had at least one good-grade embryo. Thirty percent of the patients (n = 36) had the gestational sac verified. (n = 24) The live birth rate was 20%. All of the current pregnancies ended in live births free of obstetric issues. After receiving PRP therapy, the mean EMT was 6.2 mm.

The EMT had an average increase of 0.7 mm. This distinction, nevertheless, was not statistically significant. Individually, 72 patients' EMTs went up, 42 patients' EMTs went down, but 6 patients' EMT remained the same. 12 of the 36 clinical cases of pregnancy showed an increase, whereas 24 showed a decrease in EMT. The patients did not report any negative side effects. Table 2 offers a summary of the treatment's results.

The results of the treatment cycle were contrasted with those of each patient's most recent ET cycle, which served as the control cycle. In the treatment cycle, the rates for clinical pregnancy, live births, and implantation were 12.6, 30, and 20%, respectively. The control cycle had 0% implantation, clinical pregnancy, and live birth rates. The treatment cycle had considerably greater implantation and clinical pregnancy rates than the control cycle. Age, BMI, the total number of transplanted embryos, and the quantity of good-quality embryos transferred did not differ considerably.

Table 1: The baseline characteristics of the patients

Variables	Mean±SD
Age (years)	35.7±5.2
BMI	24.2 ± 3.6
Failed IVF cycles	2.5 ± 0.8
Infertility duration	5.5 ± 2.6
Parity	
Primary	102
Secondary	18

Table 2: The results of autologous platelet-rich plasma treatment

Variables	Mean±SD
EMT final (mm)	6.1 ± 1.5
Variables	**N (%)**
β-hCGU	42 (35%)
No. of G sac	36 (30%)
EMT change	**N (%)**
Increased	72 (60)
Decreased	42 (35)
Did not changed	6 (5)

Discussion

PRP is autologous blood plasma that has been 4-5 times more heavily enriched with platelets than normal blood. Using a variety of growth factors and cytokines, such as PDGF, TGF, VGEF, EGF, fibroblast growth factor (FGF), insulin-like growth factor I, II (IGF I, II), interleukin 8 (IL8), and connective tissue growth factor, PRP can promote proliferation and regeneration (CTGF). Nowadays, PRP infusion is being widely used in numerous disciplines in medicine such as nerve injury, osteoarthritis, chronic tendinitis, bone repair and regeneration, cardiac muscles, alopecia, plastic surgery and oral surgery, but there is minimal experience in gynaecology and obstetrics. [22-25]

In assisted reproductive technology (ART) cycles, a thin endometrium is one of the major reasons for cycle cancellations. Many treatment options have been tested in this area, but not all instances have benefited from them. Low-dose aspirin, [26,27] pentoxifylline and vitamin E, [28] sildenafil citrate, [29] and oestrogen are some treatment options for thin endometrium. [30] Although PRP is frequently used in numerous clinical settings, the process for making PRP is still not standardised. As a result, the growth factor and platelet quantitation contents are not known. Critical details about the PRP used, such as cell composition, platelet concentration, and activation, were not presented in the earlier investigations. We looked for the best-known evidence to increase the efficacy of PRP and tried to provide information on PRP and the preparation procedure.

The use of PRP with a platelet concentration of roughly 1,000,000/L seems to produce the best biological results. Lower quantities have a less than ideal impact, whereas greater concentrations may paradoxically have an inhibiting effect. [31] We used an aseptic PRP preparation kit, and according to the manufacturer, the end product's platelet count

should range from 717,000 to 1,565,000/L and its WBC concentration should be between 24,000 and 37,000/L.

A high blood flow impedance of the uterine radial arteries may be the cause of the poor correlation between thin endometrium and pregnancy rate. In refractory situations, many approaches have been utilised for a while to improve ET. In this regard, platelet-rich plasma represents a novel method. Depending on how they are made and the results they provide, platelet concentrates can differ. Ehrenfest *et al*. offered four different preparations, including pure platelet-rich plasma (P-PRP)—also known as leukocyte-poor platelet-rich plasma, pure platelet-rich fibrin (P-PRF), and leukocyte- and platelet-rich fibrin (L-PRF), which is made by centrifuging blood. [32]

A minimum ET of 7 mm was recommended by Weissman *et al*. to increase pregnancy rates. [33] Richter *et al*. looked studied the correlation between fresh embryo transfer (FET) and the clinical success of in vitro fertilisation (IVF) in 1294 infertile patients. [27] For the first time in published research, Chang *et al*. [4] revealed that intrauterine injection of autologous PRP improved the ET. With a reduced cancellation rate for the PRP group, endometrial thickness was considerably larger in the PRP group compared to the control group (p 0.01). Increased implantation, pregnancy, and live birth rates with refractory thin endometrium have been suggested by Kim *et al*. [14].

However, significant randomised and controlled research are required to confirm this finding. Endometrial receptivity is controlled by dynamic and precise molecular and cellular actions involving cytokines, homeobox transcription factors, and genes. [34] Leukemia inhibitory factor (LIF), a cytokine, has been discovered to have a part in uterine prepping and embryo attachment. [35,36] LIF supplementation was used to treat implantation failure in female mice with a Lif deficiency. [37-39] PRP therapy increases the expression of LIF in endometrial stromal cells, which could improve endometrial receptivity. Moreover, it is hypothesised that PRP may help trophoblasts implant more easily. It is also crucial to note that PRP considerably outperformed traditional estradiol valerate and sildenafil citrate in non-IVF cycles in improving the ET and vascularity in individuals with unexplained infertility, according to a study by Kiran Pandey *et al*.

Whether FET boosts the pregnancy rate in IVF-ET is still up for debate. Shi *et al*. recently showed that there is no significant difference in the pregnancy outcomes between fresh and frozen embryos when transplanted to ovulatory women in a large-scale prospective randomised clinical trial (RCT). [40] There is also no conclusive data about the difference in the cumulative pregnancy rates between fresh and frozen-thawed ET cycles, according to a meta-analysis that included four RCTs. [41]

Our study's findings demonstrated PRP's effectiveness in promoting endometrial development. After two PRP injections, all participants who had a history of cancelling cycles due to thin endometrium were determined to have enough endometrial development.

There isn't much evidence in this direction right now. So, in this situation, we recommend additional clinical trials. As PRP is created from autologous blood samples, there are very few dangers associated with the process in terms of the spread of infectious diseases and immunological reactions.

Conclusion

Given the results of earlier research, autologous PRP is a novel strategy for enhancing ET and boosting pregnancy rates

in infertile women with thin, refractory endometrium. PRP is free of adverse effects because it is made from the patient's own blood. Also, because it is readily available, affordable, and administered locally, it can be a blessing for patients who are infertile. To employ PRP, more investigation in the form of extensive randomised controlled trials is required.

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