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Original Research Article

Effect of intrauterine instillation of autologous platelet-rich plasma on endometrial thickness and pregnancy outcome

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ABSTRACT

Background: Every year numerous females are detected infertile as infertility becomes a major problem in reproductive lives, worldwide. The purpose of this study is to evaluate the effectiveness of intrauterine infusion of autologous platelet-rich plasma (PRP) in infertile women undergoing frozen embryo transfer cycles with suboptimal endometrium.

Material and Methods: A total of 61 women between 22 to 40 years of age were included in the study. Based on hysteroscopy findings they were divided into group A (Normal hysteroscopy findings) and group B (Abnormal hysteroscopy findings). Intrauterine instillation of autologous PRP was done in women having an endometrial thickness of ≤ 7 even after giving Estradiol valerate therapy. After 48 and 72 hours of PRP infusion, an increase in EMT was evaluated. Embryo transfer was performed when the endometrium reached an optimal pattern in terms of thickness, appearance, and vascularity.

Result: EMT increased significantly ($p < 0.05$) in Group B compared to Group A. Between both groups, there were no apparent changes in CPR, IR, or LBR ($p < 0.001$). Overall, clinical pregnancy and LBR increased respectively.

Conclusion: Autologous platelet-rich plasma increases endometrial thickness and success rate in pregnancy after PRP administration in patients with low EMT undergoing IVF treatment and patients having two or more previous IVF failures.

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1. Introduction

Infertility among adults is a major problem causing mental and social distress. According to World Health Organization (WHO) report, approximately 8-10% of couples worldwide experience infertility problems during their reproductive lives.¹ Every year, numerous women are detected infertile due to various reasons such as ovulatory disorders, pelvic adhesions, endometriosis, bilateral tubal occlusion,

hyperprolactinemia, and acquired tubal abnormalities. Advancement and usage of assisted reproductive technology (ART) like in-vitro fertilization (IVF) have helped to combat this problem to a certain extent.²

A phenomenon that is found to be associated with the less probability of implantation of an embryo is the low endometrial thickness (EMT). It is observed that low endometrial thickness (< 7 mm) at the end of the follicular phase is negatively associated with the benefit of IVF treatment. It accounts for affecting 5% of the women undergoing IVF treatment.³

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Multiple treatment methods are suggested to increase the endometrial thickness. A Few such methods are Platelet-rich plasma (PRP) treatment, stem cell therapy, low-dose Human chorionic gonadotropin (HCG) administration, and Granulocyte colony-stimulating factor (G-CSF) treatment.⁴ Under PRP therapy, the platelet-rich plasma of autologous blood is administered to the patient. This PRP is 4-5 times more concentrated with platelets than blood.⁵ Multiple studies had demonstrated that administering PRP conjugated with Hormone Replacement Therapy (HRT) prior to embryo transfer enhances the likelihood of conception.⁶

Autologous PRP is the stimulator of many growth factors such as vascular endothelial growth factors (VEGF), epidermal growth factor (EGF), platelet-derived growth factor (PDGF), and transforming growth factor (TGF), fibroblast growth factor (FGF), insulin-like growth factor I, II (IGF1, II), connective tissue growth factor (CTGF), and cytokines. Hence it increases endometrial thickness, resulting in successful embryo implantation.⁷ Platelets also activate peripheral blood mononuclear cell (PBMC), which releases IL-10. IL-10 is a cytokine involved in tissue regeneration.⁸ A significant concentration of PDGF-AB, PDGF-BB, and TGF- were found in PRP solution during the therapy.⁹

PRP therapy is a low-cost, low-risk treatment module and advancement in the field of this therapy will result in increased benefits in ART. Our study had shown improvement in endometrial thickness and pregnancy rates after PRP administration in patients with low EMT undergoing IVF treatment.

2. Materials and Methods

The objective of this study was to evaluate the efficacy of intrauterine infusion of PRP on endometrial thickness and safety in women undergoing infertility treatments. The secondary objective was to study the implantation rate and clinical pregnancy rate.

2.1. Ethics

The protocol for this study was approved by the Ethics Committee of the Nirmal Hospital Pvt. Ltd. (Reg.No: ECR/390/INST/GJ/2013/RR-19)

This study was a prospective single-arm self-controlled trial conducted from June 2018 to June 2021 at Morpheus Shivam International IVF center and Shivam hospital, Shiv Complex, Bhatar, Surat, Gujarat.

2.2. Study design

All infertile women attending Morpheus Shivam International IVF Center for IVF were started on oral Estradiol valerate from day 1 of their menses in a dose of 6 to 8 mg/day; the amount was progressively increased to

12 mg/day in divided doses as needed. Serial transvaginal ultrasound examinations were done by using a transvaginal probe of 5 to 9 MHz, on Voluson E (Voluson, GE Healthcare, India) starting from day 7 or 8, and repeated as required to measure the endometrial thickness. Serum E2 level should be >250ng/ml.

For patients who did not respond to oral therapy, transdermal estrogen (17-β Estradiol) has been given. If ET still remains ≤7 mm then that patients were included in the study.

Patients included in this study were divided into two major groups based on hysteroscopy findings:

Group A: Patients having endometrium thickness <7mm with normal hysteroscopy findings.

Group B: Patients having endometrium thickness <7mm with abnormal hysteroscopy i.e. having polypectomy, adhesiolysis, and endometritis.

After obtaining written informed consent from the selected women, PRP was done in the stimulated cycle on the day of the trigger in both groups. Endometrial thickness was measured after 48 and 72 hours of the PRP treatment. Patients who did not show response in the form of increased thickness were submitted for repeat PRP in the next cycle.

3. Methods for PRP preparation

20 ml of blood was drawn from all the patients enrolled in the study and blood was divided equally into four test tubes for PRP preparation. These test tubes were pre-treated with 0.5 ml of Sodium Acetate as the anticoagulant. Blood was mixed well and kept in a centrifuge machine. The samples were then centrifuged at 1200 rpm for 12 minutes. Following the centrifugation, the buffy coat and the plasma (supernatant) were taken off the test tubes and added to a single test tube followed by a centrifuge of 2500 rpm for 15 minutes. The supernatant was discarded, and only 0.4 ml of plasma platelet pellet was kept, these pellets were dissolved with a pipette which helps in the activation of the platelet. The Platelet concentration was determined by making smears from a few prepared samples that were selected randomly showing 5×10^5 to 7×10^5 platelets/ml of blood.

3.1. The procedure of PRP instillation

Patients were asked to drink water to make the bladder partially full then under all aseptic precautions per speculum examination was done to see the direction of the cervix and then the external os was cleaned with a normal saline swab. An insulin syringe containing 0.1 ml of air was linked to an IUI catheter before 0.4 ml of PRP solution was drawn into the syringe. Under Trans-abdominal 2D Ultrasound Samsung R7 (5 MHz convex probe) guidance IUI catheter was introduced just beyond internal os and instillation of PRP was done. USG guide was used to avoid wrong

passage, inadvertent damage to the endometrial lining, and accidental touching of the fundus of the uterus. After instillation patients were asked to remain on the table for 10 minutes and post-procedure antibiotics were also given. Endometrial thickness was measured with 2D ultrasound Samsung R7 (7MHzTVS probe). ET was measured in the sagittal plane of the uterus to see the whole length of the endometrial lining including the cervical canal and the same was also measured after 48 and 72 hours. Embryo transfer has differed in a few patients who did not show response in form of increased thickness and were submitted for repeat PRP in the next cycle. When EMT reached more than 7mm, a daily Injection of progesterone 100 mg intramuscular (Inj. Progesterone 100mg) was started, and embryo transfer was performed on day 3 or 5 as per embryo quality, after the assessment based on the Istanbul consensus (Alpha Scientists in Reproductive Medicine and ESHRE Special Interest Group of Embryology, 2011).

Luteal phase support after embryo transfer was given with intramuscular injections of progesterone 50mg/day and vaginal progesterone. Serum β -HCG was measured two weeks after embryo transfer, and if adequate levels were achieved than TVS was performed after another two weeks to confirm clinical pregnancy. Estradiol was also continued during the luteal phase until 10 weeks of pregnancy. Patients were followed until the delivery.

Positive beta HCG rate and clinical pregnancy rates were calculated, and the women with positive pregnancy tests were followed up to see the pregnancy outcomes. Data collected were analyzed using the Graph pad prism software version 9. Paired t-test was used, and a P value of <0.01 was considered to be statistically significant.

4. Results

In this study total of 61 patients were included. Out of these 44 patients were in group A having normal hysteroscopy findings, and 17 patients were in group B having abnormal hysteroscopy findings. The average age of patients in group A was 33.3 years and it was 36.87 years in group B. The BMI of all patients in both groups was comparable. The average years of infertility were 6.5 years in group A and 8.5 years in group B. In group A, three previous IVF attempts failed only in one patient, nine patients had two failures, and 13 patients just one failure. In group B, there was 1 case where three previous IVF attempts failed, 7 cases where it failed twice, and 7 cases where it failed just once.

As per Table 2 and Figure 1 Mean increase in EMT was significantly increased following PRP administration ($p < 0.001$) with an average increase of 1.62 mm (24.96% rise) after 48 hours and 2.42mm (37.28% rise) after 72 hours of PRP instillation in the normal hysteroscopy group and an increase of 2.61 mm (43.5% rise) after 48 hours and 3.28 mm (54.66% rise) after 72 hours in abnormal hysteroscopy group.

5. Discussion

The incidence of thin endometrium varies from 1% to 2.5% in ART.^{5,6} The most common pathological causes of thin endometrium may include Asherman syndrome, history of uterine surgery, infection, and radiation.⁶ This research was conducted to determine the efficacy of autologous PRP in the study groups. Most of the studies have excluded patients having thin endometrium with abnormal hysteroscopy findings and cases in which operative hysteroscopy was done. But in this study, we have included all of these patients in group B and the results were compared for the outcome in both the groups. Adequate endometrial thickness is the main factor for implantation and pregnancy. Women with persistent thin endometrium often do not undergo embryo transfer. Although several methods have been adopted for endometrial preparation like low dose Aspirin, Pentoxifylin, Low molecular weight heparin, Sildenafil, Vaginal estrogens, Nitro-glycerine patch, etc., but the success rate is insignificant mostly. Recently, local infusion of G-CSF is introduced, which is a cytokine that stimulates endometrial proliferation.⁵ However, PRP infusion stimulates proliferation and regeneration of endometrium with a large number of growth factors and cytokines.⁷ In the present study Autologous platelet-rich plasma was infused, which contains 5 to 6 times more concentration of platelets / ml of plasma and its effect was observed.

In this study, 80% of patients have shown a significant increase in endometrial thickness, hence successful embryo transfer could be completed in the same cycle. Patients who did not show an increase in endometrial thickness were subjected to repeat PRP in the next cycle and they were excluded from the study to check secondary outcomes. In both the study groups after embryo transfer, 50% of the patients conceive successfully and our results are in accordance to the previous studies. Though, Poor response to PRP infusion cannot be explained fully, but the literature says severe damage to the basal layer may be responsible for it. Various factors influence the yield of PRP such as the drawing of blood; centrifugation speed and time, Temperature of centrifugation, and use of anticoagulants, therefore the yield of PRP can also influence the results of PRP therapy.¹⁰

For the first time, Chang reported the efficacy of intrauterine infusion of PRP for endometrial growth in women with thin endometrium. In that trial, PRP was infused in 5 Women with adequate endometrium who had a poor response to conventional therapy during the FET cycle. The proper response to treatment was reported in all of them, and normal pregnancy was reported in 4 women(Changetal.,2015).⁹

GokalpOner (Department of Obstetrics and Gynaecology, Kayseri Acibadem Hospital, Turkey) has published three case reports of hysteroscopic guided

Table 1: Characterisation of the study groups according to the parameters

Parameters	Group A (Normal Hysteroscopy) (N=44)	Group B (Abnormal Hysteroscopy) (N= 17)	P P Value
	Mean \pm SD		
Age (years)	33.3 \pm 5.3	36.87 \pm 4.69	0.018
BMI (kg/m ²)	27.2 \pm 7.34	28.63 \pm 5.53	0.470
Duration of infertility (years)	6.5 \pm 2.88	8.25 \pm 3.37	0.047
Previous IVF failure (attempt)	1.55 \pm 0.97	1 \pm 0.85	0.044
Basal Pre PRP E2 (ng/dl)	553 \pm 428	546.02 \pm 430.6	0.954

Table 2: Comparison of EMT in both the study groups

Study groups	Pre PRP EMT	EMT after 48 hrs of PRP	EMT after 72 hrs of PRP	Outcomes		P value
				Mean and % increase in EMT after 48 hrs of PRP	Mean and % increase in EMT after 72 hrs of PRP	
Group A Normal Hysteroscopy (N=44)	6.49 \pm 0.72	8.11 \pm 0.78	8.91 \pm 1.35	1.62mm & 24.96%	2.42mm & 37.28%	<0.05
Group B Abnormal Hysteroscopy (N=17)	6.00 \pm 0.58	8.61 \pm 2.25	9.28 \pm 2.47	2.61mm & 43.5%	3.28mm & 54.66%	
P value	0.01	0.20	0.46			

Table 3: Showing various parameters during the IVF cycle

	Normal Hysteroscopy Group A (N=44)	Abnormal Hysteroscopy Group B (N=17)
Embryo transfer performed on day 3	25	8
Embryo transfer performed on day 5	19	9
Fresh et	12	9
Thaw et	32	13
Total embryo transferred	D3-79 D5-19	D3-22 D5-19
Average embryo Transferred per cycle	2.81 \pm 0.84	2.06 \pm 1.11

Table 4: Characterization of study groups according to parameters

Study groups	Positive b-HCG		Negative b-HCG		Clinical pregnancy		Live birth		Live birth rate per embryo	Implantation rate per embryo
	No.	%	No.	%	No.	%	No.	%	%	%
Group A Normal Hysteroscopy (N=44)	24	54.55%	20	45.45%	22	50%	30	47%	24.8%	25.2%
Group B Abnormal Hysteroscopy (N=17)	10	58.82%	7	41.18%	9	52.94%	10	40%	24.4%	26.82%

PRP instillation and Showed good results in endometrial thickness.¹¹

DR Meenu et al., analyzed hysteroscopic instillation of PRP in the endometrial junction to improve endometrial thickness for embryo transfer in the subsequent cycle. As such, 75% of the patients achieved an endometrial thickness of 7mm or more, underwent an embryo transfer, and out of that 50% of the patients conceived. In our study, the patients who did not show an increase in endometrial thickness were subjected to repeat PRP in the next cycle.⁸

Research conducted by Maryam Eftekhari et al., found that pregnancy rate and LBR were 20%. The implantation and clinical pregnancy rates were 12.7% and 30%, respectively, and the difference was statistically significant. The average increase in the EMT was 0.6 mm compared with the EMT of their previous cycle. However, this difference was not statistically significant. Further, the EMT of 12 patients increased (mean difference: 1.3mm), while that of seven patients decreased (mean difference: 0.7mm); the EMT of one patient did not change. There were no adverse effects reported by the patients who were treated with Autologous PRP.¹² Zadehmodarres et al. in a pilot study revealed the efficacy of PRP on endometrial growth. Adequate endometrial growth was found in all the participants after two PRP infusions in all patients who had a history of the cycle cancellation due to the thin endometrium.¹³ PRP is an effective, safe, low-cost, easy treatment with no major side effects to improve endometrial thickness and help to reduce the cycle cancellation rate in IVF practice.

6. Conclusion

Autologous PRP instillation in the uterine cavity significantly increases endometrial thickness in patients with endometrium and also found to be effective in reducing the cycle cancellation rate. PRP Instillation is found more efficacious in women having abnormal hysteroscopic finding (group B) as compared to women having normal hysteroscopic finding (group A). Increased in endometrial thickness was statistically significant ($P < 0.05$). There was no significant difference in pregnancy outcome (clinical pregnancy rate and LBR) in both groups. There was no significant difference in CPR, IR, and LBR among both groups ($p < 0.001$). Overall, clinical pregnancy and LBR increased respectively. No adverse reactions were reported. From the present study it is reported that PRP is an effective, safe, affordable and convenient method with no major side effects to improve endometrial thickness and help to reduce the cycle cancellation rate in IVF practice.

7. Supplementary Materials

The following are available online at www.mdpi.com/xxx/s1, Figure S1: title, Table S1: title, VideoS1: title.

8. Author Contributions

All authors had contributed equally in this research.

9. Institutional Review Board Statement

The protocol for this study was approved by the Ethics Committee of Nirmal Hospital Pvt Ltd (Reg.No:ECR/390/INST/GJ/2013/RR-19) at Surat, Gujarat, India

10. Informed Consent Statement

Informed written consent was obtained from all subjects involved in the study.

Abbreviations


CPR: Clinical pregnancy rate; MDPI: Multidisciplinary Digital Publishing Institute; LBR: Live birth rate; PRP: Platelet-rich plasma; IR: Implantation rate; IVF: ET in-vitro fertilization-embryo transfer; FET Frozen embryo transfer; HRT: Hormone replacement therapy; TVS: Trans vaginal sonography.

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