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Effectiveness of Autologous Platelet-Rich Plasma Therapy in Women with Repeated Implantation Failure: A Randomized Clinical Trial

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Abstract

Background: Platelet-rich plasma (PRP) therapy has been shown to enhance tissue regeneration by expressing several cytokines and growth factors (GFs). This study investigated the effect of intrauterine infusion of PRP as a non-invasive autologous GF on pregnancy outcomes in women with repeated implantation failure.

Materials and Methods: This randomized clinical trial was conducted to compare the pregnancy rates between two groups of women who were candidates for the frozen-thawed embryo transfer with a history of two or more implantation failures. The PRP group (n=33) was treated with hormone replacement therapy+0.5 cc to 1 cc PRP infused into the uterine cavity two days before the embryo transfer. The control group (n=33) was only treated with hormone replacement therapy. The endometrial preparation process was done similarly in both groups. The chemical, clinical, and ongoing pregnancy, and implantation rates were compared between the two groups.

Results: Our results showed that the chemical pregnancy rate was not statistically higher in the PRP group in comparison with the control group (36.4 vs. 24.2%). In addition, the clinical pregnancy, ongoing pregnancy, and implantation rates were higher in the PRP group than the control group; however, the difference between the two groups was not statistically significant.

Conclusion: Administration of intrauterine PRP before embryo transfer in women with repeated implantation failure (RIF) does not affect assisted reproductive technology (ART) outcomes (registration number: IRCT2016090728950N3).

Keywords: Embryo Implantation, Platelet-Rich Plasma, Pregnancy Outcome

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Introduction

Despite many advances in the treatment of infertile couples in recent years, some couples still suffer from repeated implantation failure (RIF). RIF refers to the occurrence of unsuccessful embryo transfers after undergoing two to six cycles of *in vitro* fertilization (IVF). Many different factors, such as embryo quality, endometrial receptivity, and immunological factors, can be involved in this situation. Several methods have been proposed to manage RIF, including blastocyst transfer, assisted hatching, hysteroscopy, endometrial scratching, and immune therapy. However, a technique with the most impact is still discussed (1).

Previous studies have examined the immune system's role in the recurrent reproductive failure. Most of them focused on the role of peripheral blood markers more than the uterus environment. They showed the active role of local immune cells at the implantation site in the endometrial receptivity (2, 3). Our previous study showed that a dose of 0.5 cc (300 µg/ml) intrauterine injection of granulocyte colony-stimulating factor (GCSF) by the use of an IUI catheter immediately after ovarian puncture improved the pregnancy rate in women with a RIF history (4). While, the GCSF is naturally produced in the reproductive system and exacerbates the proliferation and differentiation of neutrophilic granulocytes, which acts on decidual cells macrophages and ultimately affects the implantation rate (5, 6). In addition, some studies showed differences in the effect of platelet-rich plasma (PRP) in women with thin endometrium undergoing IVF or intracytoplasmic injection (ICSI) with frozen embryo transfer cycles (7-11). Other studies have suggested PRP treatment as a low-cost and non-invasive approach that allows natural concentrations of autologous growth factors (GFs). This

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treatment has been widely tested in various medical fields to examine its potential for improving tissue regeneration (12, 13). PRP is defined as an autologous blood plasma fraction with a platelet concentration of about four-five times higher than the baseline. Intra-platelet chemical mediators, through the promotion of angiogenesis and the onset of cellular regeneration by mitotic mediators of mesenchymal cells, trigger the onset of regeneration (14).

PRP is prepared from fresh whole blood, and stored in acid citrate dextrose solution A (ACD-A) anticoagulant. After separating the various blood components, platelet concentrations are increased, and finally, by activating the platelets, the cytokines and GFs become bioactive and released 10 min after clotting (15). PRP can improve the regeneration of various tissues by expressing several cytokines and GFs. Studies have investigated the role of intrauterine injection of autologous PRP in suboptimal endometrium and have shown that PRP can improve embryo transfer (ET) and vascularization by releasing cytokines and GFs such as vascular endothelial GF, transforming GF, platelet-derived GF, and epidermal GF. These factors regulate cell migration, proliferation, and differentiation while increasing extracellular matrix accumulation (16).

The present study was designed to investigate the effect of intrauterine infusion of PRP as a non-invasive autologous GF on the pregnancy outcomes of women with a RIF history.

Materials and Methods

The study protocol was approved by the Yazd Research and Clinical Center for Infertility Ethics Committee, Yazd, Iran (IR.SSU.RSI.REC.1395.16) and the Iranian Registry of Clinical Trial (IRCT) (IRCT2016090728950N3). In addition, written informed consent was obtained from all couples before participating in this study. The present study was conducted at the Yazd Reproductive Sciences Institute, Yazd, Iran.

Primary outcomes: chemical and clinical pregnancy. Secondary outcomes: ongoing pregnancy and implantation rate.

Sample size

The sample size was estimated to be a minimum of 72 (36 in each group) by considering the significance level of 95%, the power of 80%, ongoing pregnancy of our center, and considering a 30% difference based on the study by Chang et al. (15), and used the following formula:

$$n = \frac{(Z_{1-\alpha/2} - Z_{1-\beta})^2 \times [P_1(1-P1) + (P_2(1-P2)]}{(P1-P2)^2}$$

In this randomized clinical trial, women aged 18-42 years with a history of two or more implantation failures candidates for a frozen-thawed embryo (s) transfer who were referred to the Research and Clinical Center for Infertility, Yazd Reproductive Sciences Institute, Yazd, Iran, between September 2016 and January 2017 were enrolled. The participants were randomized into two groups (PRP and control) with the use of a computer-generated randomization list. All women with immunological, hormonal, or hematological disorders, such as thrombocytopenia (platelet count<1050/ul) or Hb<10 g/dl, as well as congenital abnormalities and uterine abnormalities (congenital or acquired) were excluded. To conceal allocation, the interventions were sealed in serially numbered, opaque envelopes of equal appearance and weight and then distributed to participants.

All participants underwent hormone replacement therapy (HRT) for endometrial preparation, as described below. Each woman was orally prescribed 6 mg/day of Estradiol Valerate (Tablet 2 mg, Aburaihan Co., Tehran, Iran) from the second day of the menstrual cycle. From the 13th day of the menstrual cycle, the periodic vaginal ultrasonography was done to measure the endometrial thickness by an infertility fellowship. When the endometrial thickness reached 8 mm, 400 mg vaginal progesterone (Cyclogest; Actavis, UK Limited, England) was administered twice daily. Estradiol and progesterone were administrated in all women until two weeks after embryo transfer. In the case of positive chemical pregnancy, HRT continued until the 10th week of gestational age.

In the PRP group, 0.5-1cc of PRP was infused into the uterine cavity two days before embryo transfer. The control group was only treated with HRT. For PRP preparation, 8.5 ml of peripheral venous blood was taken into a syringe containing 1.5 ml of anticoagulant acid citrate A (ACD-A) solution (Aria Mobna kit, Iran) and centrifuged at 1600 g for 10 minutes at ambient temperature. After that, the plasma layer and buffy coat were transferred into another tube and centrifuged again at 3500 g for 5 minutes to obtain 1.5 ml PRP with a concentration of four to five times the platelet compared to expect. Then, 0.5-1 cc of PRP was infused into the uterine cavity in the PRP group, and the participants were asked to remain lying down for 10 minutes.

In all participants, three days after the progesterone administration, 1-2 cleavage embryos were transferred one day after thawing using a Labotect catheter (Labotect, Gotting, Germany). The serum beta human chorionic gonadotropin (βhCG)>50 IU/L, 12 days after embryo transfer, was considered a positive chemical pregnancy. The observation of fetal heart activity two weeks after the positive βhCG was considered a positive clinical pregnancy. Ongoing pregnancy was defined as a continuation of pregnancy after the 12th week of gestation, and the implantation rate as the number of gestational sacs per 100 embryos transferred.

Statistical analysis

Data were analyzed using the SPSS software (version 20.0, SPSS Inc., Chicago, Illinois, USA). The Chi-Square ($\chi 2$), One-way ANOVA, and Student's t tests were used to evaluate the relations between variables. $P<0.05$ was considered statistically significant.

Results

Initially, 72 women were eligible to enter the study. Six women were excluded due to dissatisfaction to continue participating in the study. Finally, 66 women with RIF history participated in this study and were randomized into two equal groups: the PRP group and the control group (Fig.1). No significant differences were observed between the two groups in age, etiology of infertility, and embryo transfer quality (Table 1).

Table 1: Demographic characteristics of participants in two study groups

Variables	PRP group (n = 33)	Control group (n = 33)	P value
Age (Y)	32.48 ± 4.95	33.45 ± 3.45	0.36*
Number of embryo transfer	1.96 ± 0.58	2.18 ± 0.46	0.10*
Etiology of infertility			0.66**
PCOS	6 (18.2)	7 (21.2)	
Male factor	6 (18.2)	8 (24.2)	
Unexplained	6 (18.2)	7 (21.2)	
Tubal factor	(12.1)	4 (12.1)	
Endometriosis	3 (9.1)	0 (0)	
Ovarian failure	3 (9.1)	4 (12.1)	
Combined	5 (15.2)	3 (9.1)	
Endometrial thickness (mm)	8.92 ± 1.00	9.25 ± 1.16	0.21*
Quality of embryo transfer			0.74**
A	5 (15.2)	6 (18.2)	
B	28 (84.8)	27 (81.8)	

PRP; Platelet-rich plasma, PCOS; Polycystic ovarian syndrome, *; Students' t test, and **; Chi-square test.

Our results showed that the rate of chemical pregnancy was higher in the PRP group than the control group (36.4 vs. 24.2%), but the difference was not significant (P=0.28). In addition, the rates of clinical pregnancy, ongoing pregnancy, and implantation were also non significantly higher in the PRP group than the control group (Table 2).

Table 2: Comparison of ART outcomes between the two study groups

ART outcomes	PRP group (n=33)	Control group (n=33)	P value
Chemical pregnancy rate	12 (36.4)	8 (24.2)	0.28
Clinical pregnancy rate	11 (33.3)	8 (24.2)	0.41
Ongoing pregnancy rate	8 (24.2)	6 (18.2)	0.54
Implantation rate (%)	8/65 (12.30)	12/72 (16.66)	0.47

All data presented as n (%). Chi-square test. PRP; Platelet-rich plasma and ART; Assisted reproductive technology.

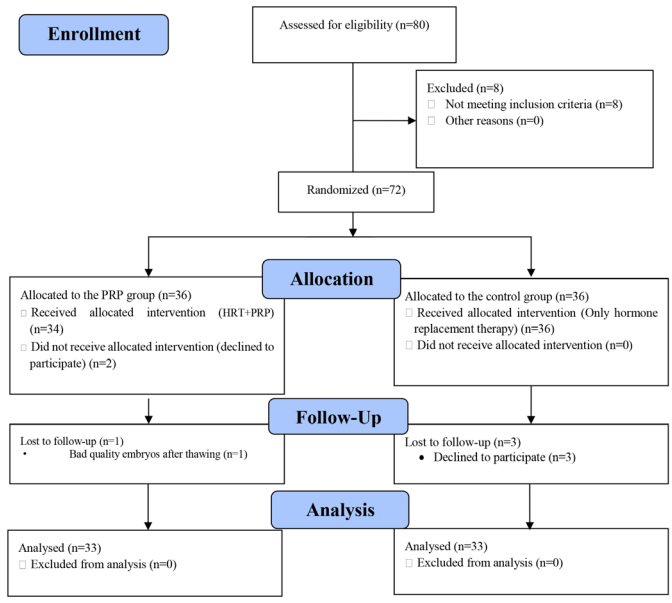

Fig.1: The study CONSORT flow diagram. PRP; Platelet-rich plasma, HRT; Hormone replacement therapy.

Discussion

In this study, we evaluated the endometrial receptivity after PRP administration in the RIF women according to the hypothesis that the local infusion of PRP due to the several GFs and cytokines may improve endometrial receptivity and implantation rate in the women with a history of RIF. Our present findings did not suggest that the PRP therapy is clinically effective for women receiving frozen-thawed embryo (s) transfer.

Tehraninejad et al. (17), in a study including 85 women with RIF and normal endometrial thickness, showed that the PRP is not a good option for treatment of women with a RIF history and normal endometrial thickness candidates for embryo transfer; similar to this study endometrial thickness was normal (more than 7 mm) in all of our participants. PRP was more effective in improving embryo transfer cycles in women with thin endometrium in our previous study. We showed PRP improved the endometrial thickness and pregnancy rates in frozen -thawed embryo (s) transfer with a thin endometrium (7)

In contrast to our findings, the results of a meta-analysis showed that an intrauterine administration of PRP increases the clinical pregnancy rate in women with a frozen-thawed embryo transfer. They introduced PRP as an alternative treatment strategy in patients with thin endometrium and RIF history (18).

The role of autologous PRP injection in the suboptimal endometrium has been evaluated in several studies. It is estimated that PRP, through the release of cytokines and GFs, the regulation of cell migration, adhesion, proliferation, and differentiation, as well as the enhancement of extracellular matrix accumulation, may effectively improve endometrial transfer and vascularity (16).

There are a few studies that reported the positive effect of PRP on the endometrial growth and pregnancy outcomes (15, 19, 20). Chang et al. (15) undertook for the first time a pilot study of PRP in 5 patients with thin endometrium. 48-72 hours after PRP infusion, they observed an increase of endometrial thickness in all the patients (>7 mm on the progesterone administration day), and all of them were pregnant. Garcia-Velasco et al. (21) reported that the use of autologous PRP improved the endometrial transfer in women with refractory endometrium. Also, we have previously evaluated the effect of an intrauterine administration of PRP in women with thin endometrium during a frozen embryo transfer cycle We observed that the of 0.5-1 cc PRP was associated with a significantly higher rate of implantation and clinical pregnancy rates in a 83 women (7).

Farimani et al. (22) reported a positive pregnancy in a 45-year-old woman with a primary infertility history after two IVF cycles failure candidate of the donor eggs. She was treated with an intrauterine injection of autologous PRP 24 hours before embryo transfer. Evaluating the effect of an intrauterine administration of PRP on the pregnancy rate, Nazari et al. (23) reported 90% pregnancy rate in a study of 20 women with a history of RIF, also 16 of them had clinical and ongoing pregnancies at the time of the study. They concluded that the use of PRP effectively improved pregnancy outcomes in RIF patients, that was inconsistent with Obidniak et al. (24) study. They concluded that this method should be considered perspective, safe, and cost-effective in treating these patients. Also, our previous study showed that intrauterine injection of 0.5 cc of GCSF before embryo transfer improved pregnancy outcomes in patients with a history of implantation failure (4). GCSF, as a hematopoietic lineage-specific cytokine, is naturally synthesized in the reproductive system and stimulates the proliferation and differentiation of hematopoietic cells of the neutrophilic granulocyte lineage, which affects the macrophages of the decidual cells, ultimately resulting in implantation (5, 6).

Further studies are recommended regarding the population under investigation, the time frame evaluated, and the comparative studies approach between drugs and autologous preparations to envisage effective therapeutic alternatives for RIF.

Conclusion

Administration of intrauterine PRP before embryo transfer in women with recurrent implantation failure does not affect asssisted reproductive technology (ART) outcomes.

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Authors' Contributions

M.E.; Study conception and Design. M.E., N.N., P.Kh.; Conducted the procedures and Data analysis. Further, all authors participated in the review of the literature and drafting the manuscript, approved the final version of the manuscript, and take responsibility for the integrity of the data.

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