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Autologous platelet-rich plasma infusion improves clinical pregnancy rate in frozen embryo transfer cycles for women with thin endometrium

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Abstract

Background: Adequate thickness of the endometrium has been well recognized as a critical factor for embryo implantation. This was a prospective cohort study to investigate the benefits of platelet-rich plasma (PRP) for women with thin endometrium who received frozen embryo transfer (FET) program in a larger number of patients and explore the underlying mechanism.

Methods: In this study, we investigated the effects of PRP in women with thin endometrium in FET program. 64 patients with thin endometrium (<7 mm) were recruited. PRP intrauterine infusion was given in PRP group during hormone replacement therapy (HRT) cycle in FET cycles.

Results: After PRP infusion, the endometrium thickness in PRP group was 7.65 ± 0.22 mm, which was significantly thicker than that in control group (6.52 ± 0.31 mm) ($P < .05$). Furthermore, PRP group had lower cycle cancellation rate when compared to control group (19.05% *vs.* 41.18%, $P < .01$). The implantation rate and clinical pregnancy rate in PRP group were significantly higher than those in control group (27.94% vs 11.67%, $P < .05$; 44.12% vs 20%, $P < .05$, respectively). PRP blood contained 4 folds higher platelets and significantly greater amounts of growth factors including platelet-derived growth factor (PDGF)-AB, PDGF-BB, and transforming growth factor (TGF)-β than peripheral blood ($P < .01$).

Conclusions: PRP plays a positive role in promoting endometrium proliferation, improving embryo implantation rate and clinical pregnancy rate for women with thin endometrium in FET cycles.

Abbreviations: FET = frozen embryo transfer, HRT = hormone replacement therapy, PDGF = platelet-derived growth factor, PRP = platelet-rich plasma, TGF = transforming growth factor.

Keywords: platelet-rich plasma, pregnancy rate, thin endometrium

1. Introduction

Adequate thickness of the endometrium is crucial for successful embryo implantation. Although, meta-analysis performed by Kasius et al demonstrated that receiver operating characteristic (ROC) curve could not determine a definite cut-off value for endometrium thickness to predict a successful pregnancy,[1] a trend of decreased pregnancy rate was observed in women with thin endometrium when compared with women with thick endometrium. Thin endometrium significantly increases the risk of embryo implantation failure,[2] as the pregnancy rate in women with ≤6 mm of endometrial thickness was only 29.43%.[3]

Nowadays, therapies for thin endometrium include long-term administration of estrogen,[4] low-dose of aspirin,[5] vitamin E supplement,[6] vaginal sildenafil citrate application,[7] electro-acupuncture[8] and transvaginal endometrial perfusion of granulocyte colony stimulating factor (G-CSF).[9] However, even with these treatment options, a number of women with thin endometrium still have no response. Thus, there is a need for an alternative treatment for patients with thin endometrium.

Platelet-rich plasma (PRP) derived from fresh whole blood and has anti-inflammatory and pro-regenerative functions.[10] After the activation of the platelets in PRP, growth factors as vascular endothelial growth factor (VEGF), transforming growth factor (TGF), platelet-derived growth factor (PDGF) and epidermal growth factor (EGF) are actively secreted and transformed into their bioactive forms within 10 minutes after clotting.[11] These growth factors are known to regulate cell functions such as attachment, migration, proliferation, differentiation and promote extracellular matrix accumulation.[12] Therefore, PRP has been widely used for various therapeutic areas, for example, promoting healing process in orthopedics, ophthalmology and healing therapies.[13–16] But there was no study of applying it to promote endometrial proliferation. In 2015, we first reported a

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The author(s) of this work have nothing to disclose.

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pilot study of PRP in 5 patients with thin endometrium who had poor response to standard therapies,[17] in which all 5 women had successful pregnancy and 4 women had babies except 1 patient suffered from abortion. However, the mechanisms underlying the effects of PRP on endometrium proliferation remains unclear.

We hypothesized that PRP intrauterine infusion would effectively increase endometrial thickness and improve frozen-thawed embryo transfer (FET) outcomes. In this prospective study, we were intended to further investigate the benefits of PRP for women with thin endometrium who received frozen embryo transfer (FET) program in a larger number of patients and explore the underlying mechanism.

2. Materials and methods

2.1. Study design and patients

This was a prospective cohort study, which was conducted in Reproductive Medicine Center, Sixth Affiliated Hospital of Sun Yat-Sen University (Guangzhou, China) from July 2015 to July 2016. The study was approved by Institutional Review Board of Sixth Affiliated Hospital of Sun Yat-Sen University (NO: 2014ZSLYEC-007S). Patients with thin endometrium who were going to receive FET, and met the following inclusion criteria were enrolled in this study:

(1) age younger than 40 years old, and basal serum FSH <10IU/L;
(2) with cancellation history of embryo transfer due to thin endometrium (<7 mm) in hormone replacement therapy (HRT) cycles;
(3) no obvious intrauterine adhesion in the diagnostic hysteroscopy;
(4) no submucosal uterine myoma or endometrial polyps;
(5) no history of hematological disorders (e. g., leukopenia, thrombasthenia et al);
(6) have at least 2 frozen good-quality blastocyst-stage embryos.

Patients were excluded if she had a history of pelvic cancer, severe endometriosis, and adenomyosis. Endometrial thickness was measured with vaginal ultrasound at its thickest part in the longitudinal axis of the uterus and was performed by the same investigator using a computerized vaginal ultrasound (Aloka Prosound SSD-3500, Japan). This investigator was blind to the conditions of patients. Thin endometrium was defined as the endometrium thickness <7 mm on the day when progesterone was given in HRT cycles.

2.2. Endometrial preparation

All patients were treated with HRT protocol, the endometrial priming started on the day 2 to 3 of each patient's menstrual cycle, and E_2 valerate (Bayer Schering Pharma, France) was orally administered with a start dose of 6 mg/d. The dose of E_2 valerate was increased according to the endometrium response. The daily maximal dose was 12 mg. When endometrial thickness failed to reach over 7 mm, Patients were consulted to make decision to cancel the cycle, proceed to a new FET cycle or undergo embryo transfer regardless of thin endometrium. Whether or not to receive PRP treatment depends on the preference of the patients. As PRP administration was an experimental treatment, the choice of the patients did not affect their following treatment. Patients receiving PRP treatment had PRP intrauterine infusion on the 10^{th} day and the day when progesterone was given in HRT cycle with written informed consent. The other patients who did not receive PRP intrauterine infusion were included in control group.

2.3. Embryo transplantation

Two good-quality blastocyst-stage embryos were transferred to all patients. The criterion for embryo quality was consulted from the embryo morphology assessment by the Istanbul consensus workshop, blastocysts scored as Grade 3 or 4 were considered as good-quality.[1] After embryo transplantation, the luteal phase was supported by daily intramuscular injection of 40 mg progesterone, combined with 200 mg vaginal progesterone soft capsules every night. Serum human chorionic gonadotropin (hCG) level was tested 14 days after embryo transfer. Vaginal ultrasonography was performed 35 days after transfer in cases of a biochemical pregnancy, and a clinical pregnancy was defined as the presence of an intrauterine fetal heart beat. Implantation rate was determined by the number of gestational sacs over the total number of embryos transferred.

The primary endpoint is endometrial thickness. The second endpoints are implantation rate and clinical pregnancy rate.

2.4. PRP preparation and infusion

PRP was prepared from autologous blood as reported previously.[10] Approximately 15 mL of venous blood was collected in a syringe prefilled with 5 mL anticoagulant solution (ACD-A). The blood was then centrifuged immediately at 300 X g for 10 minutes at 18°C. Three layers were formed after centrifugation, red blood cells at the bottom, a cellular plasma in the supernatant, and a buffy coat layer in the middle. The upper 2 layers were transferred into a new sterile tube and then centrifuged at 700 X g for another 15 minutes at 18°C. After discarding about 3-quarters of the supernatant, 0.5 to 1 mL of PRP was pipetted from the bottom of tube. The PRP was activated by adding a mixture of thrombin powder (25 IU/mL; Sigma-Aldrich, St. Louis, MO) and calcium chloride (20mmol/mL; Kalmia, Korea United) at 18°C. The number of platelets was measured in whole blood and PRP by an automatic blood tester (Siemens Advia 2120i, Siemens Healthcare Diagnostics Inc, Erlangen, Germany). A volume of 0.5-1 mL PRP was infused into uterine cavity with Tomcat catheter in PRP group each time.

2.5. ELISA

The levels of PDGF-AB/BB and TGF-β in the whole blood and PRP were measured by Enzyme-linked Immunosorbent Assay (ELISA) following the instruction of manufacturer (R&D systems, Minneapolis, MN). The samples were diluted 10 to 20 times accordingly before detection to make sure the concentrations in the assay range (under 2000 or 1000 pg/mL of upper detecting limit).

2.6. Statistics

Statistical analysis was carried out by using the Statistical Package for the Social Sciences version 17.0 (SPSS IBM Corp). Continuous variables were presented as mean ± SD and assessed by independent samples *t*-test. The number and percentage are presented as n (%), and the numerical data were analyzed using the Chi-squared test and Fisher exact test and presented as percentages. $P < .05$ was considered statistically significant.

3. Results

Seventy-five women were assessed for eligibility, 11 of them were excluded as they did not meet the inclusion criteria. A total of 64 patients with thin endometrium (<7 mm) and previously canceled FET cycles were recruited, 34 patients receiving PRP uterine infusion were defined as PRP group, 8 of them had repeated cycles because they canceled embryo transfer for thin endometrium in the first treatment cycle. So there were 42 cycles in PRP group, the cycle cancellation rate was 19.05% (8/42). The other 30 patients without PRP infusion were included in the control group, 21 patients had to cancel transfer cycles because of thin endometrium. The cycle cancellation rate was 41.18% (21/51), which was significantly higher than that in PRP group ($P<.05$). The general characteristics of these patients were summarized in Table 1. Age, BMI, gravidity, cause of infertility, number of prior HRT cycles and endometrial thickness in prior cycles were comparable between the 2 groups ($P>.05$).

After PRP treatment, endometrium thickness in PRP group was 7.65 ± 0.22 mm, which was significantly thicker than that in control group (6.52 ± 0.31 mm, $P<.05$). In PRP group, 15 patients had clinical pregnancy, and 4 of them had 2 gestational sacs with vaginal ultrasound test, the clinical pregnancy rate was 44.12% (15/34), and implantation rate was 27.94% (19/68). 6 patients in control group had clinical pregnancy, 1 of them had twin pregnancy, the clinical pregnancy rate was 20% (6/30), and implantation rate was 11.67% (7/60), which were significantly lower than those in PRP group ($P<.05$) as shown in Table 2. Miscarriage rate and ectopic pregnancy rate were not significantly different. No patients were reported to have transmission/immunogenic reactions or infection.

In order to explore the underlying mechanisms of PRP, we analyzed plasma cyto-components in PRP and whole blood. The average platelet concentration in PRP was 4.21-folds higher than that in whole blood (889.42 ± 64.41x10^3/μL vs 211.37 ± 46.20x10^3/μL, $P<.01$). The concentrations of PDGF-AB, PDGF-BB, and TGF-β were much higher in PRP compared with whole blood (33 ng/ml vs. 10 ng/mL, 6.6 ng/ml versus 1.5 ng/mL, 30 ng/mL versus 13 ng/mL, respectively, all $P<.01$) (Fig. 1).

Table 1

Characteristics of participants with blastocyst-stage embryo transplantation*.

	PRPgroup (n = 34)	Control group (n = 30)	P
Age, years	34.77 ± 0.75	32.64 ± 1.70	.17
BMI, kg/m^2	22.42 ± 0.42	22.39 ± 0.80	.49
Duration of infertility, years	3.57 ± 1.82	3.71 ± 1.66	.52
Menstrual cycle, day	29.40 ± 0.29	28.51 ± 0.43	.82
Basal FSH, IU/L	5.91 ± 1.77	6.36 ± 1.84	.36
Basal LH, IU/L	4.80 ± 1.19	4.31 ± 1.32	.77
Basal E2 (pg/ml)	38.50 ± 10.93	40.70 ± 11.22	.85
No of embryo transplanted	2	2	
Cause of infertility, n (%)			
Pelvic/tubal factors	21 (61.76)	17 (56.67)	1.25
PCOS	5 (14.71)	4 (13.33)	.97
Endometriosis/Adenomyosis	4 (11.76)	4 (13.33)	.64
Other causes†	4 (11.76)	5 (16.67)	.73
No. of prior HRT cycles	2.15 ± 1.09	1.97 ± 1.33	.35
Endometrial thickness in prior cycles	**6.32 ± 0.54**	**6.39 ± 0.72**	**.51**

PRP = platelet-rich plasma.
* Values are given as mean±SD or number (percentage), unless indicated otherwise.
† unknown causes of infertility, as uterine factors or cervical factors.

Table 2

Cycle characteristics and outcomes of FET cycles in PRP and control groups.

	PRPgroup (n = 34)	Control group (n = 30)	P
Endometrium thickness	7.65 ± 0.22	6.52 ± 0.31	.013
Cycle cancellation, n (%)	8 (19.05)	21 (41.18)	.022
Cycle non-cancellation, n (%)	34 (80.95)	30 (58.82)	.022
Clinical pregnancy, n (%)	15 (44.12)	6 (20)	.036
Implantation, n (%)	19 (27.94)	7 (11.67)	.018
Miscarriage, n (%)	0 (0)	1 (16.70)	.33
Ectopic pregnancy, n (%)	1 (7.7)	0 (0)	.99

FET = frozen embryo transfer, PRP = platelet-rich plasma.

4. Discussion

In this study, we investigated the effects of PRP in FET program and demonstrated that PRP uterine infusion significantly improved endometrial thickness, embryo implantation, and clinical pregnancy rate.

Repeated uterine curettage leads to injury and chronic inflammation of the endometrium. The functional impairment or mechanical damage is responsible for decreased endometrial regenerative activities and increased implantation failure. In our study, endometrium of all the 64 patients did not respond to hormone treatment, leading to cancellation of embryo transfer. The commonly accepted minimal endometrial thickness is 7 mm at the end of follicular phase, so we used a cut-off of 7 mm to diagnose thin endometrium.[18] In the present study, endometrial thickness in PRP group was significantly thicker than the control group, which resulted in less cycle cancellation. Besides, the clinical pregnancy rate and implantation rate were both improved in the PRP group. In patients without the problem of thin endometrium, the average endometrial thickness was about 10 mm, and the clinical pregnancy rate was 50% to 55% in FET Cycles in our centre. Although the endometrial thickness in patients before PRP treatment was lower than that in the healthy subjects, significantly increased endometrial thickness by PRP was still observed, which further resulted in improved clinical pregnancy rate.

Platelet activation leads to a release of the aforementioned growth factors from α-granules to promote cartilage matrix synthesis, cell growth, migration and phenotype changes.[19] PDGF may exert positive effect on tissue regeneration through its mitogenic activity and synergy with TGF-β1. TGF-β is the most important growth factor released by platelets during healing and contributes to proliferation of fibroblasts, narrow stem cells, and osteoclasts captivity. Recent animal study by Jang et al shown that intrauterine administration of autologous PRP stimulated and accelerated regeneration of the endometrium and also decreased fibrosis in a murine model of damaged endometrium.[20] In our study, significantly higher concentrations of PDGF-AB, PDGF-BB, and TGF-β were observed in PRP solution than those in peripheral blood. It is suggested that PRP with high concentration of growth factors and cytokines can stimulate the mitogenesis and proliferation of endometrial cells or endometrial stem cells, and then activate endocrine-paracrine pathways for improving the endometrial response to promote embryo implantation and pregnancy. Besides, it has been reported that platelets might activate peripheral blood mononuclear cell (PBMC), which releases IL-10, an anti-inflammatory cytokine involved in tissue regeneration.[20]

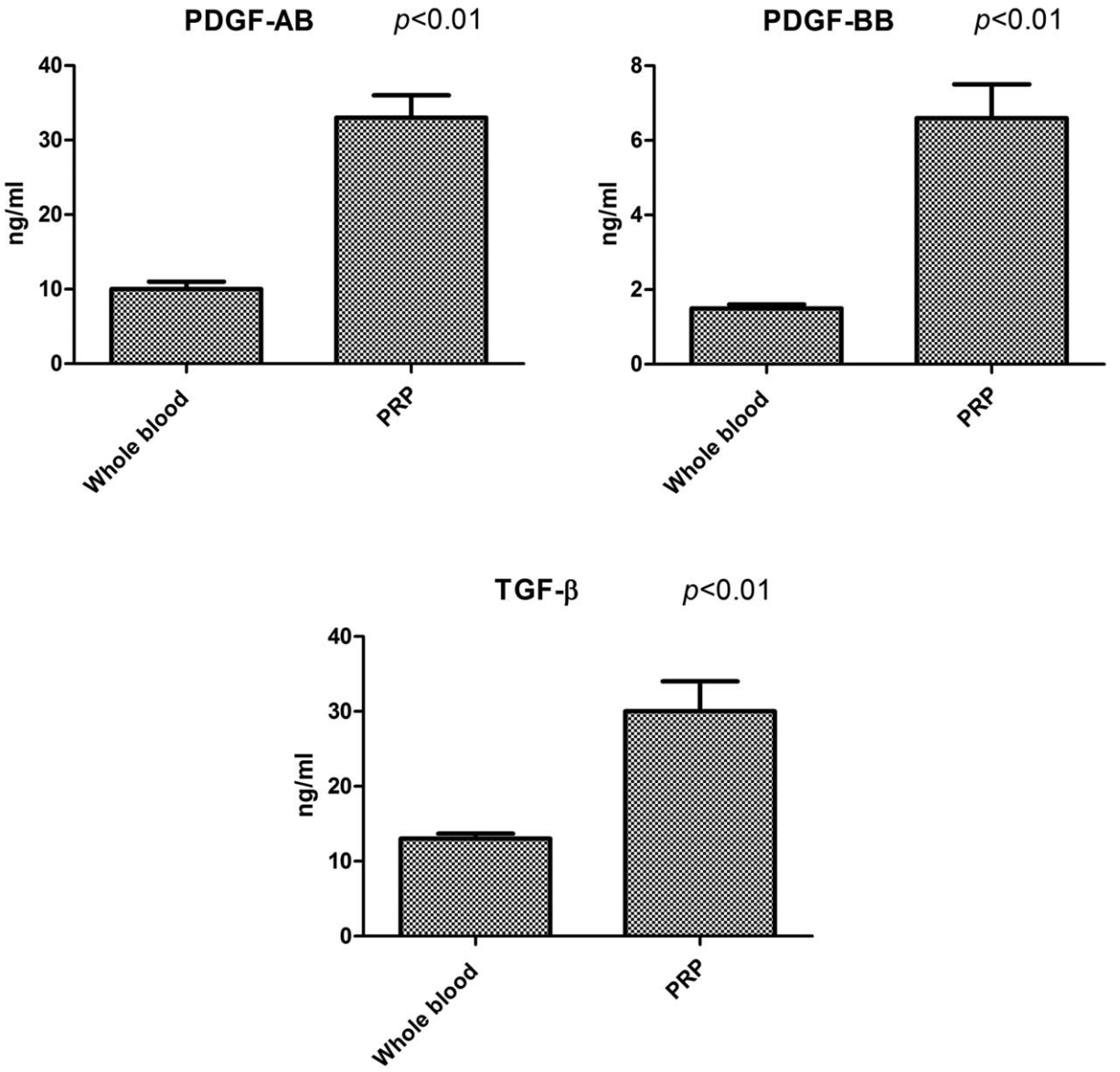

Figure 1. The concentrations of PDGF-AB, PDGF-BB and TGF-β in PRP and whole blood. The data are expressed as the mean ± SD. PDGF = platelet-derived growth factor, PRP = platelet-rich plasma, TGF = transforming growth factor.

In 2015, we first reported intra-uterine infusion of PRP increasing endometrium thickness in 5 patients with thin endometrium who were unresponsive to regular treatments. Activated PRP promoted the migration of human primary endometrial epithelial cells, endometrial stromal fibroblasts, endometrial mesenchymal stem cells (MSC) and bone marrow-derived MSC. These data provide an initial ex-vivo proof of principle for the use of autologous PRP to promote endometrial regeneration in Asherman's syndrome and a thin endometrial lining. To the best of our knowledge, this is the first prospective study to investigate the effectiveness of PRP intrauterine infusion for thin endometrium in FET cycle.

However, different protocols of PRP preparation elicit different responses. Amable demonstrated that PRP concentration varied due to relative centrifugal force (RCF), temperature and time, and concluded that with temperature at 18°C, the RCF at 300 × g in the first time and 700 × g at the second time resulted in the highest platelet yield, purity and recovery without growth factor secretion throughout sample manipulation.[10] Therefore, double-step centrifuge technique, with 300 × g and 700 × g of RCF respectively, was applied to concentrate the platelets in our study. Over 4-fold enrichment of platelets was obtained and obvious positive effectiveness of PRP was observed as for endometrial thickness and relevant clinical outcomes.

As PRP is derived from autologous blood, it prevents problems such as transmission and immunogenic reactions. Based on the observation in thousands of patients treated with the PRP after oral-maxillary surgery, the PRP treatment was considered safe in patients.[21] Adverse effects such as infection are rare. In this study, no patient had any noticeable side effects after PRP infusion.

However, our study was a prospective study and recruitment mainly depended on patients' preference. It has the instinct bias of comparison of the study design. Meanwhile, the sample size was relatively small. Moreover, the underlying mechanism and biological pathways still needs to be further investigated. More patients and good quality design of RCTs are also needed to confirm our observations.

In conclusion, our data indicate that PRP efficiently improved the endometrium proliferation, implantation rate, and clinical pregnancy rate. The high concentrations of various growth factors in PRP were proposed to be the possible mechanism. PRP intrauterine infusion could be an effective alternative treatment for patients with thin endometrium in FET cycle.

Author contributions

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