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Original Article

Can autologous platelet rich plasma expand endometrial thickness and improve pregnancy rate during frozen-thawed embryo transfer cycle? A randomized clinical trial

Maryam Eftekhar [a], Nosrat Neghab [a, *], Elham Naghshineh [b], Parisa Khani [a]

[a] Reasearch and Clinical Center for Infertility, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
[b] Department of Obstetrics and Gynecology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran

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ABSTRACT

Objective: One of the important aspects involved in achieving optimal outcomes after assisted reproductive treatment (ART) is the endometrium. Some cycles are cancelled due to inadequate endometrial growth in ART. In this clinical trial, we evaluated the effectiveness of platelet-rich plasma (PRP) in the treatment of thin endometrium.

Materials and methods: In this randomized clinical trial, 83 women with poor endometrial response to standard hormone replacement therapy (HRT) (endometrium thickness < 7 mm) in the 13th day of the cycle in a frozen-thawed embryo transfer (FET) were entered in two groups. In the PRP group (n = 40), in addition to HRT, 0.5–1 cc of PRP was infused into the uterine cavity on the 13th day of HRT cycle. The control group (n = 43) was only received HRT. If endometrial thickness failed to increase after 48 h, PRP infusion was repeated in the same cycle. When the endometrium thickness reached ≥7 mm, embryo transfer was done. Finally, endometrial thickness, chemical, clinical, and ongoing pregnancy rates were compared between two groups.

Results: Endometrial thickness increased significantly to 8.67 ± 0.64 in PRP group than in controls (p = 0.001). This increase was higher in women who conceived in PRP group (p value: 0.031). The implantation rate and per-cycle clinical pregnancy rate were significantly higher in PRP group (p = 0.002 and 0.044, respectively (p = 0.002).

Conclusion: PRP may be effective in improving the endometrial growth, and possibly pregnancy outcomes in women with a thin endometrium.

Introduction

It was established that pregnancy rate was affected negatively by endometrial thickness <7 mm and thin endometrium resulted in significantly lower implantation and pregnancy rates and also correlated with a higher risk of miscarriage. Therefore, the thin endometrium is an extremely poor factor that interferes with an ongoing pregnancy [1,2].

Immunological mechanisms such as growth factors, hormones, and cytokines, which are produced by decidual cells have very important and crucial role in the implantation in the endometrium [2].

Some frozen-thawed embryo transfer (FET) cycles are cancelled due to the thin endometrium, and there is no conventional protocol for this condition. Hormonal manipulation like an extended dose of estrogen or improving endometrial perfusion by low dose aspirin, Pentoxifylline and vitamin E, Sildenafil, and new modalities like Granulocyte colony-stimulating factor (G-CSF) are used for endometrial expansion [3]. Combined treatment with pentoxifylline and vitamin E, 6–9 months before embryo transfer, significantly improved the pregnancy rate by increasing endometrial thickness [4].

Intrauterine perfusion with -G-CSF has been used for thin endometrium, but there isn't any proved evidence in this treatment [5–7].

* Corresponding author. University of Medical Sciences and Health Services, Yazd Research and Clinical Centre for Infertility, Bouali Ave, Safaeyeh, Yazd, Iran.
E-mail addresses: eftekhar@ssu.ac.ir (M. Eftekhar), nosrat20@yahoo.com, nosratneghab@ssu.ac.ir (N. Neghab), Naghshineh@med.mui.ac.ir (E. Naghshineh), pkhani55@ssu.ac.ir (P. Khani).

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Regenerative medicines with stem cells or intrauterine infusion of platelet-rich plasma (PRP) are other new modalities that have recently been suggested for the treatment of thin endometrium [3,6]. PRP is blood plasma prepared from fresh whole blood that has been enriched with platelets. Platelets have positive effects on local tissue repair and contain a significant amount of growth factors that stimulate proliferation and growth like vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), platelet-derived growth factor (PDGF), transforming growth factor (TGF), and other cytokines [6,8]. For more than a decade PRP has been used in several medical conditions in ophthalmology, orthopaedics, surgery and, wound healing in injectable or gel form, it stimulates regeneration of the soft tissues such as fat, skin, and mucosa as well as the hard tissues like tendons and bones [6,9]. But it's efficacy in endometrial growth has not been fully clarified. In this study, we attempted to evaluate the efficacy of autologous PRP in the treatment of thin endometrium in FET cycles and pregnancy rate.

Method and materials

In this randomized clinical trial, 83 women with thin endometrium referred to Yazd Research and Clinical Center for Infertility, Yazd, Iran between September 2016 and January 2017 were enrolled.

The research proposal was approved by the Ethics Committee of Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran and registered in Iranian registry for clinical trials (IRCT2016090328950N2). Also, a written informed consent was obtained from all women for participating in this trial.

Our inclusion criteria were women candidated for FET because of poor endometrial response (endometrium thickness < 7 mm) to standard hormone replacement therapy (HRT) in the 13th day of the cycle in FET cycles with age between 18 and 42 years. All women with haematological, immunological or hormonal disorders chromosomal and genetic abnormalities and congenital or acquired uterine abnormalities were excluded.

According to the table of random numbers, participants in the study were divided into two groups: The case group (n = 33) treated with HRT + intrauterine infusion of 0.5–1 cc PRP on the 13th day of HRT cycle. The control group (n = 33) was only treated with increased received HRT. Hysteroscopic examination was performed before the cycle if it had not been done previously.

For all women, a basic transvaginal sonography was done in 2nd day of FET cycle and estradiol valerate tablet (Aburaihan Pharmaceutical Co., Tehran, Iran) 2 mg three times daily was initiated. In the 13th day of cycle, trans vaginal sonography was done again. Endometrial thickness was measured at its thickest part in the longitudinal axis of the uterus by infertility fellowship. When the endometrial thickness was <7 mm, the same physician measured it repeatedly for 2 times to confirm thin endometrium, and the average value of the 2 different measurements was recorded. Then the women with endometrial thickness <7 mm entered in the study in two to groups (PRP group and control group) randomly according to table of random numbers with computerized software. The estradiol was increased to 10 mg in both groups. In PRP group, 0.5–1 ml PRP was infused intrauterine with IUI (intra uterine insemination) catheter (Takwin, Iran) the and in controls, estradiol was continued for another 2 days. Endometrial thickness was measured after 48 h in both groups. If endometrial thickness didn't reach 7 mm or above, the second dose of PRP was infused in PRP group and estrogen continued in controls then they were assessed after 48 h.

PRP was prepared from autologous blood using a modified two-step centrifuge process. On the 13th day of the menstrual cycle in PRP group, 8.5 ml of peripheral venous blood was drawn in the syringe that contained 1.5 ml of Acid Citrate A Anticoagulant solution (ACD-A) (Arya Mabna Tashkhis, Iran) and centrifuged immediately at 1600 g for 10 min. The blood was divided into three layers: red blood cells at the bottom, cellular plasma in the supernatant and a buffy coat layer between them. The plasma layer and buffy coat were collected to another tube and centrifuged again at 3500 g for 5 min to obtain 1.5 ml PRP with 4–5 times more concentrated and 2000 lymphocyte [6].

The FET cycle was cancelled if the endometrial thickness didn't reach to 7 mm or above in both groups until 17th cycle day, ultimately. When endometrial thickness reached 7 mm or above, vaginal suppository progesterone (Cyclogest; Actavis, the UK limited, England) 400 mg twice a day was prescribed for 3 days then embryos in cleavage state are transferred in both groups.

Estradiol and progesterone were continued after the transfer till the 12th week of gestational age if pregnancy occurred. Vitrification and warming were performed according to vitrolife instruction. Warmed embryos were transferred to a culture media and evaluated 1 day later.

The number of transferred embryos depended on the embryo quality and the women's age. The criteria for embryo quality were used from the embryo morphology assessment according to Dokras et al. criteria, and cleavage-stage embryos scored as grade A, B, C, and D. The grade D embryos were not transferred. Grade A embryo: no fragmentation with equal sized homogenous blastomeres, grade B embryo: <20% fragmentation with equal sized homogenous blastomeres, grade C embryo: 20%–50% fragmentation with unequal sized blastomeres, grade D embryo:> 50% fragmentation with unequal sized blastomeres [10].

The implantation rate was defined as the ratio of gestational sacs to the number of embryos transferred The chemical pregnancy was defined as serum B-hCG ≥50 IU/L after 14 days from embryo transfer, and the clinical pregnancy as the presence of a gestational sac with heart beat identified by ultrasound 5 weeks after the embryo transfer The abortion rate as clinically recognized pregnancy losses before 20 weeks of gestation and ongoing pregnancy as pregnancy continued after 20 weeks.

The primary outcome was the expansion of endometrial thickness in case and controls and in women who conceived and didn't conceive. The secondary outcome was pregnancy rate.

Statistical analysis

The Statistical Package for Social Sciences 16(SPSS, SPSS Inc, Chicago) was used to perform all the statistical analyses. The Chi-square ($\chi 2$) test was used to analyze nominal variables. Normally distributed Kolmogorov–Smirnov test parametric variables were tested by independent Student's t-test. Non normally distributed metric variables were analyzed by Mann–Whitney U test. $P < 0.05$ was considered statistically significant. Values were expressed as the mean ± standard deviation (SD) unless otherwise stated.

Results

Eighty five women in FET cycle with thin endometrium in the 13th cycle were enrolled in this study. Two of them had not inclusion criteria and excluded, so, in the first step of study 83 women participated in two groups: PRP group (n = 43) and control group (n = 40). Of them, 10 women in the control group and seven in the PRP group had no embryo transfer due to the persistent thin endometrium (Fig. 1). In PRP group 23 women need only one and 10 need two PRP infusion to achieve ET ≥ 7 mm.

There were not significant differences between two groups in age and infertility type (Table 1).

The cycle duration, number of transferred embryos, embryo quality, and 1st endometrial thickness in 13 days of the cycle (D1)

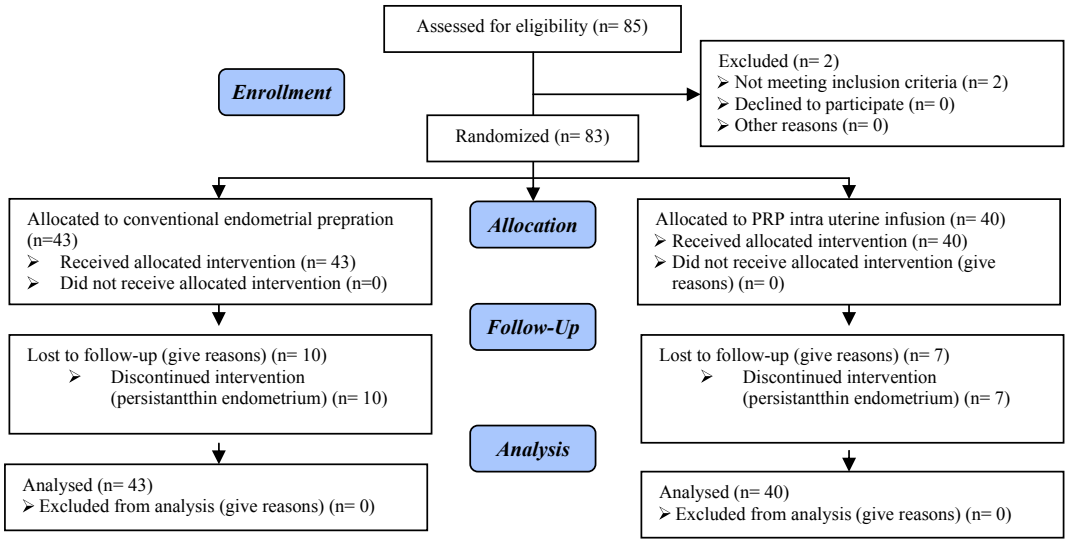

Fig. 1. Study flow chart.

showed no significant differences among groups but the endometrial thickness after intervention (D2) was significantly difference (Table 2).

When we divided the groups into two subgroups according to whether the examined women conceived, we found that the endometrium expanded significantly from 6.12 ± 0.35 to 8.80 ± 0.75 mm in conceived PRP group and from 6.06 ± 0.59 to 8.54 ± 0.53 mm in non conceived women (p-value = 0.031) and in controls from 6.10 ± 0.20 to 8.06 ± 0.40 and from 6.05 ± 0.17 to 8.04 ± 0.27 (p value: 0.062) respectively (Table 3).

The embryo data and the clinical outcome of the study groups are compared in Table 4. The implantation rate and per-cycle clinical pregnancy rate were significantly higher in PRP group (Table 4).

Cycle cancellation rate was lower in PRP group but not significant (7 in PRP group and 10 in controls, p = 0.061).

Discussion

Optimal endometrium thickness is one of critical factors for successful embryo implantation. Consequently, endometrium preparation has been considered a crucial step for embryo transfer.

Table 1
Basal characteristics of participants in two groups.

	PRP group (n = 40)	Control group (n = 43)	P-value
Age (years)	31.98 ± 2.26	32.40 ± 2.63	0.199[a]
Infertility type, n (%)			
Primary	29 (72.5)	31 (72.50)	
Secondary	11 (27.5)	12 (27.90)	0.967[a]
Etiology of infertility n (%)			
Male factor	13 (32.5)	6 (14.0)	0.021[b]
Female factor			
PCOS	0 (0)	3 (7.0)	
DOR	1 (2.5)	1 (2.3)	
Tubal factor	0 (0)	5 (11.6)	
Endometriosis	0 (0)	4 (9.3)	
Mixed (MF + FF)	12 (30.0)	9 (20.9)	
Unexplained	14 (35.0)	15 (34.9)	

PRP: platelet-rich plasma; DOR: Diminished ovarian reserve; PCOS: Polycystic ovary syndrome; MF + FF: Male factor + Female factor.
[a] Student *t*-test.
[b] Chi–Square test.

Many scientists are interested in investigating the physiological and pathological processes of the thin endometrium, and different treatment strategies have been explored during last few years, including extended estrogen administration, vasoactive medicines such as low-dose aspirin, sildenafil citrate, pentoxifylline-tocopherol, and GCSF intrauterine infusion. However, even by these remedies, a small number of women remained unresponsive and thus recurrently cancelled ET cycles or failed in IVF-ET due to the thin endometrium. Effective treatment for thin endometrium is still a challenge that has not been solved. New therapeutic approaches of increasing endometrial thickness would be urgently required [11].

When tissue damage occurs, the first cells reach at the site of injury are platelets. Anuclear platelets contain several types of granules involved in coagulation, inflammation, atherosclerosis, antimicrobial host defence, and angiogenesis. These a-granules contain many growth factors, including platelet-derived growth factor, transforming growth factor beta, vascular endothelial growth factor, insulin-like growth factor, fibroblast growth factor, epithelial growth factor, and keratinocyte growth factor, as well as many cytokines, chemokines, and resulting metabolites. PRP contains high levels of these autologous growth factors. Many studies have suggested that inflammation, postoperative blood loss, infection, and narcotic requirements can be reduced by PRP and acceleration in osteogenesis and wound and soft tissue healing [12,13].

Efficacy and safety of autologous PRP have been reported in many fields of medicine but there are few clinical trials to determine the role of PRP [11]. The molecular mechanisms of PRP therapy in the endometrial proliferation are not well understood at present. We hypothesized that intra uterine infusion of PRP can expand thin endometrium and improve pregnancy rate.

PRP is quite a new treatment used for the improvement the endometrial thickness in women with a thin endometrium. It seems that PRP is safe because of autologous nature derived from patient's own blood.

Chang et al. administered an intrauterine infusion of PRP in infertile women with a thin endometrium. In 4 women from five with thin endometrium and poor response to conventional therapy during FET cycles normal pregnancy was reported.

Some studies show that the pregnancy occurs when endometrium reaches more than 7 mm [2].

Table 2
Cycle characteristics in the transferred cycle (n = 33/each group).

	PRP group	Control group	P value
Cycle duration (mean ± SD)	18.27 ± 1.06	18.57 ± 0.93	0.090*
No. of Transferred embryos (mean ± SD)	2 ± 0.433	1.93 ± 0.609	0.622*
Embryo quality			
A	20	22	0.342
B	41	38	0.243
C	12	10	0.233
Endometrial thickness, (mm) at			
D1	6.09 ± 0.47	6.15 ± 0.37	0.555
D2	8.67 ± 0.64	8.04 ± 0.27	0.001

PRP: platelet-rich plasma; D1(1st endometrial thickness in the 13th day of cycle). D2(endometrial thickness after intervention).
Embryo quality: A: no fragmentation with equal sized homogenous blastomeres, B: <20% fragmentation with equal sized homogenous blastomeres, C: 20%–50% fragmentation with unequal sized blastomeres, D:>50% fragmentation with unequal sized blastomeres.

Table 3
Endometrial thickness in women who conceived and didn't conceive in PRP and control group (D2).

	Women who conceived (chemical pregnancy) (n = 22)	Women who didn't conceive (n = 44)	p-value
Endometrial thickness in PRP group (D2)	8.80 ± 0.75	8.54 ± 0.53	0.031
Endometrial thickness in controls (D2)	8.06 ± 0.40	7.97 ± 0.53	0.062

D2(endometrial thickness after intervention).

Table 4
The outcomes of embryo transfer cycles.

	PRP group (n = 40)	Control group (n = 43)	P-value*
Implantation rate	21%	9.37%	0.002
Chemical pregnancy			
Per transfer	14 (42.4%)	8 (24.2%)	0.191
Per cycle	14 (35.0)	8 (18.0)	0.091
Clinical pregnancy			
Per transfer	13 (39.4%)	6 (18.2%)	0.102
Per cycle	13 (32.5)	6 (14.0)	0.044
Ongoing pregnancy			
Per transfer	11 (33.3%)	6 (18.2%)	0.260
Per-cycle	11 (27.0)	6 (14.0)	0.127
Abortion rate	3 (9%)	2 (6%)	0.613

p value $\leq$ 0.05 is significant.

Chang et al. reported the efficacy of intrauterine infusion of PRP for endometrial growth in women with thin endometrium for the first time. Five patients with a history of the thin endometrium (on the day of hCG administration) were recruited ed in the study. PRP was infused into the uterine cavity on the 10th day of FET cycle. If endometrial thickness failed to increase 72 h later, PRP infusion was done 1–2 times in each cycle. When the endometrium thickness reached >7 mm embryos were transferred. Successful endometrial expansion and pregnancy were observed in all the patients after PRP infusion [6].

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 cycle cancellation due to the thin endometrium [14].

Conclusion

It seems that PRP can expand endometrial thickness with recruiting growth factor to the endometrium. PRP is a safe procedure, with minimal risks of infectious disease transmission and immunological reactions since it is made from autologous blood samples. In our study which is the first randomized clinical trial PRP group had lower cancellation rate and higher pregnancy rate but statistics were not significant. We suggest further clinical trials with more population in this milieu.

Conflict of interest

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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