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Effects of autologous Platelet-Rich Plasma treatment on thin endometrium in patients undergoing frozen-thawed embryo transfer cycles

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ABSTRACT

Objective

The objective of this study is to determine whether the treatment of a thin endometrium with autologous platelet-rich plasma (PRP) prior to an embryo transfer increases positive reproductive outcomes in cycles where implantation failure is attributable to a thin endometrium, during the endometrial preparation cycles.

Material and Methods

This is a cohort, prospective and interventional study, carried out in a private facility. Women between 32 and 45 years old were included, with a history of two or more failed IVF/ICSI (In vitro fertilization/Intracytoplasmic sperm injection) cycles and a thin endometrium (<7 mm), the cycles were with their own ovules or from donors not older than 28 years, with or without alterations in the seminal samples of their partners.

Twenty-five women were enrolled in this study, the patients were treated with an intrauterine infusion of autologous PRP 2 or 3 times during the menstrual cycle on days 8, 10 and 12 of endometrial preparation for their frozen-thawed embryo transfer (FET) cycle, and Embryo Transfer (ET) was performed 3 to 5 days after the final autologous PRP infusion. A total of 25 patients underwent FET.

Results

Of 25 patients included in this study, the mean endometrial thickness after PRP treatment was 8.6 mm. The average increase in endometrial thickness was 1.76 mm this difference was statistically significant when compared with the previous cycles for each patient, respectively. The results of the cycles with PRP infusion treatment were compared with the previous results obtained in the same group of patients. The implantation, clinical pregnancy, and live birth rates (LBR) in the PRP treatment cycle were 24.59, 56, and 48%, respectively. Implantation, clinical pregnancy, and LBR in the control cycle were 0%. Implantation, clinical pregnancy, and LBR were significantly higher in the PRP treatment cycle than in the control cycle. Age, BMI, number of embryos transferred, and number of good quality embryos transferred were not significantly different.

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Conclusions

The present study revealed a noticeable improvement in endometrial thickness after the PRP treatment considering the history of the patients, however, more studies are needed to elucidate the molecular basis of the PRP action mechanism on the endometrium and to support the results obtained and generate more solid evidence on the beneficial effect of autologous PRP treatment on the thin endometrium.

KEYWORDS: Thin endometrium, platelet-rich plasma (PRP), recurrent implantation failure, frozen-thawed embryo transfer, endometrial receptivity, endometrial thickness.

MANUSCRIPT

Background

Since the first introduction of IVF (in vitro fertilization) and ET (embryo transfer), technology has evolved rapidly and the pregnancy rate with FET (frozen-thawed embryo transfer) has increased significantly. However, thin, or damaged endometrium remains an unresolved problem in the treatment of infertility patients. Several treatments have been tried to restore endometrial receptivity, including the administration of exogenous estrogens, vitamin E, vaginal sildenafil citrate, and pentoxifylline (1). Patients with a thin endometrium who do not respond to the abovementioned treatment do not foresee many options and it is known that an endometrium with a thickness of less than 7 mm is not optimal for an embryo implantation and it is associated with a low pregnancy rate (2). Recently, progress has been made in the treatment of damaged or thin endometrium with the use of therapies that promote cell proliferation, including stem cell therapy (3). However, there are still unresolved issues related to the safety of using bone marrow-derived stem cells (4).

Autologous PRP is an alternative known for its safety. Analogous platelet products have been used since the 1970s and have become more popular since the 1990s (5). Platelets are known as the blood component that plays a crucial role in hemostasis. During the healing process, α-granules within platelets secrete growth factors, cytokines, and chemokines. These various secreted proteins have paracrine effects on myocytes (6), tendon cells (7), mesenchymal stem cells of different origins (8), chondrocytes, osteoblasts (9), fibroblasts and endothelial cells, stimulating migration, cell proliferation, angiogenesis and, consequently inducing tissue regeneration (10).

The first study on PRP for the treatment of thin human endometrium in vivo was published in 2015 (11). Four studies followed up and concluded that PRP is an influential treatment for patients with thin endometrium (12, 13, 14,15). The studies stated that autologous PRP promotes endometrial growth and improves pregnancy outcomes. However, the number of patients was small, and they did not provide enough information on the type or concentration of PRP they used. It is recognized that the effectiveness of PRP treatments can vary depending on the concentration of platelets (16). In the present study, we assessed the effect of a platelet-rich infusion treatment on the thin endometrium of patients with a history of failed IVF/ICSI cycles with respect to pregnancy rates.

Materials and Methods

We conducted a prospective and interventional cohort study. The patients were recruited from November 2017 to October 2021 at the Acapulco Institute of Reproduction and Gynecology (IREGA). Women with a history of two or more failed IVF cycles and thin endometrium in previous cycles were included in this study. The inclusion criteria were as follows: (a) age 32 to 45 years at the time of the procedure, (b) endometrial thickness <7 mm at the end of estrogen administration in FET, (c) two or more failed IVF/ICSI cycles, (d) more than two cycles of prior therapy to increase endometrial thickness, such as hysteroscopic adhesiolysis with subsequent hormone replacement therapy (HRT), the use of high-dose estradiol valerate, transvaginal administration of sildenafil , or combination of pentoxifylline with vitamin E, (f) at least one good quality frozen embryo available for transfer, and (g) signed informed consent form. The exclusion criteria were patients with an endometrial thickness greater than 7 mm at the end of estrogen administration in FET cycles, adenomyosis, endometrial uterine pathology such as polyps and submucosal fibroids, and patients with thrombophilia.

Preparation of Autologous PRP

On each day of PRP treatment administration, with the patients fasting, 18 mL of venous blood was extracted using 30 mL syringes coated with 1.25 mL of citrate-dextrose anticoagulant solution A (TERUMOBCT, USA). Blood samples were centrifuged at 1120 G for 3 min in a KITLAB Ck-12 centrifuge (DESEGO, Mexico). The buffy coat and plasma just above the buffy coat were collected, and 0.7-1.0 mL of PRP was produced and infused into the uterine cavity.

Autologous administration of PRP and ET

Intrauterine autologous PRP administration was performed in the FET cycle parallel to estrogen

administration. The patients began taking a daily dose of 4 to 8 mg of estradiol valerate (Primogyn, Bayer, Germany) from day 2 of the menstrual cycle to prepare the endometrium. The first autologous PRP infusion was performed on cycle day 10 and repeated at 3-day intervals until endometrial thickness reached 7 mm. PRP was administered into the uterine cavity using an embryo transfer catheter within 1 hour from the completion of the PRP centrifuge preparation. The syringe containing the PRP was connected to the embryo transfer catheter and the PRP was infused, this procedure was subsequently confirmed on ultrasound.

Ultrasound was used to measure endometrial thickness on day 2 of the menstrual cycle and each day of autologous PRP administration until the ET. The ET was performed 3 days after the final autologous PRP administration. When the endometrial thickness reached 7 mm or more, 50 mg of strong yellow body (HORMONA, Mexico) as progesterone were administered intramuscularly (IM), plus 20 mg piroxicam (Senosiain, Mexico) every 12 hours, until the day of the ET. The serum level of β-hCG was measured from peripheral blood 2 weeks after the ET. A follow up ultrasound was conducted to those with positive β-hCG results another 2 weeks later to confirm clinical pregnancy. Clinical pregnancy was defined as the presence of an intrauterine gestational sac. All patients received luteal support with Utrogestan, a 200mg micronized progesterone (Besins Manufacturing, Belgium) after the ET. If the transvaginal ultrasound showed a gestational sac and embryonic heart beats 4-6 weeks after the ET, luteal support was continued until week 10 of gestation. Obstetric follow up was given to clinical pregnancies up until the outcome of at least one alive newborn.

Comparison of results between treatment and previous cycles

In the same patient group variables of the previous cycles were compared with those of the PRP treatment cycle. Clinical outcomes were also compared between cycles, cycles with endometrial thickness >7mm without the need for treatment against cycles with PRP treatment. Primary results were ongoing pregnancy rate and LBR. Secondary results were implantation rate, clinical pregnancy rate, and the increase of endometrial thickness compared to the previous cycle.

Statistical analysis

The descriptive analysis was performed by the mean and standard deviation for quantitative variables and frequency, proportion was used for categorical variables. Statistical analysis of the outcome measures and associated clinical variables was performed with XLSTAT version 2020, with Student's t-test and $\chi 2$. A value of $p < 0.05$ was considered statistically significant.

Results

A total of 25 women were recruited, and all of them underwent ET. No patients were lost to follow up and all data on the 25 women were collected. The average age of the patients was 38.6 years at the time of treatment. The mean duration of infertility was 3.45 years. The mean number of failed IVF cycles was 1.83. The mean endometrial thickness on the final day of estrogen administration of the previous cycles was 6.84 mm (Table 1).

PARAMETER	PREVIOUS CYCLE (n=25)	PRP TREATMENT CYCLE (n=25)	P VALUE
Age (years)	37.11 ± 4.24	38.60 ± 4.31	0.867
BMI (Kg/m2)	26.03 ± 3.31	26.09 ± 3.21	0.974
Duration of infertility (years)	3.45 ± 3.95		-
Number of previous failed cycles	1.83±0.55		-
AMH (ng / ml)	2.69 ± 1.88	2.20 ± 1.22	0.342
FSH (mUI / ml)	6.57 ± 1.63	6.56 ± 1.70	0.769
LH (mUI / ml)	4.94 ± 1.92	5.03 ± 1.96	0.859
Infertility cause			
Female factor (n, %)	8 (32)	7 (28)	0.762
Masculine factor (n, %)	8 (32)	9 (36)	0.803
Number of oocytes retrieved.	12.31 ± 6.6	12.56 ± 6.3	0.709
Fertilization method			
IVF (n, %)	17 (68)	16 (64)	0.748
ICSI (n, %)	8 (32)	9 (36)	0.819

Table 1. Baseline characteristics of patients.

The average number of transferred embryos in each patient was 2.44 (2 or 3) in the PRP treatment group. Grading of embryos at the cleavage stage was performed using the parameters established at the Istanbul Consensus Workshop on Embryo Evaluation (17). Blastocysts were classified using the Gardner classification system.

A good grade embryo was defined as a grade I or II cleavage stage embryo with six or more cells and a blastocyst score of 3BB or higher. The ET in the morula stage was not recorded. The gestational sac was confirmed in 56% (n = 14) of the patients. One patient had a miscarriage at 9 weeks of gestational age. Another patient had a heterotopic pregnancy, with a spontaneous abortion of the intrauterine fetus at 7 weeks of gestation. The LBR was 48% (n = 12). All ongoing pregnancies resulted in live births without obstetric complications. The mean endometrial thickness after PRP treatment was 8.6 mm. The average increase in endometrial thickness was 1.76 mm, this difference was statistically significant. The results of the PRP treatment cycle were compared with the previous results obtained in the same group of patients. The implantation, clinical pregnancy, and LBR in the treatment cycle were 24.59, 56, and 48%, respectively. Implantation, clinical pregnancy, and LBR in the control cycle were 0%. Implantation, clinical pregnancy, and LBR were significantly higher in the treatment cycle than in the control cycle. Age, BMI, number of embryos transferred, and number of good quality embryos transferred were not significantly different. The PRP treatment outcomes are summarized in Table 2.

PARAMETERS	PREVIOUS CYCLE WITHOUT PRP TREATMENT (n=25)	PRP TREATMENT CYCLE (n=25)	P VALUE
Endometrial thickness on the last day of endometrial priming (mm)	6.84 ± 0.87	8.60 ± 1.12	<0.0001
Fresh/frozen cycle	4/21	0/25	0.114
Autologous/heterologous cycle	11/14	13/12	0.852
Number of transferred embryos	2.29 ± 0.53	2.44 ± 0.49	0.869
Number of good quality embryos transferred	1.8 ± 0.43	1.7 ± 0.47	0.786
Implantation rate (n, %)	0/58(0)	15/61(24.59)	<0.0001
Clinical pregnancy rate (n, %)	0/25(0)	14(56)	<0.0001
Ongoing pregnancy rate (n, %)	0/25(0)	12(48)	<0.0001
Live born rate (n, %)	0/25(0)	12(48)	<0.0001

Table 2. Comparison of results between PRP treatment and previous cycles.

Correspondingly, the results of the PRP treatment cycle were compared with the standard results of a patient group without the need for PRP treatment. Implantation, clinical pregnancy, and LBR were not significantly higher in the PRP treatment cycle compared to the traditional cycles. Age, BMI, endometrial thickness, number of embryos transferred, and number of good quality embryos transferred were not significantly different, however, there was a statistically significant difference in the proportion of autologous and heterologous compared cycles. The treatment results are summarized in Table 3.

PARAMETERS	STANDARD CYCLE WITHOUT PRP TREATMENT (n=47)	PRP TREATMENT CYCLE (n=25)	P VALUE
Age (years)	36.28 ± 4.59	38.60 ± 4.31	0.567
BMI (Kg/m^2)	26.14 ± 3.49	26.09 ± 3.21	0.969
Endometrial thickness on the last day of endometrial priming (mm)	9.05 ± 1.12	8.60 ± 1.12	0.762
Fresh/frozen cycle	7/40	0/25	<0.0001
Autologous/heterologous cycle	35/12	13/12	0.157
Number of transferred embryos	2.30 ± 0.64	2.44 ± 0.49	0.520
Number of good quality embryos transferred	1.6 ± 0.6	1.7 ± 0.47	0.701
Implantation rate (%)	37/159(23.27)	15/61(24.59)	0.237
Clinical pregnancy rate (%)	24/47(51.06)	14/25(56)	0.982
Ongoing pregnancy rate (%)	22/47(46.80)	12/25(48)	0.913
Live born rate (%)	22/47(46.80)	12/25(48)	0.913

Table 3. Comparison of results between the group of patients with PRP treatment and standard cycles with patients with a normal endometrium.

Discussion

The optimal endometrium thickness is one of the critical factors for the successful implantation of the embryo. Accordingly, the priming of the endometrium has been considered a critical step for ET.

In this study, there were no differences in other clinical characteristics, such as age, infertility duration, number of failed IVF/ICSI cycles, and embryo grading, and number of embryos transferred according to pregnancy outcomes. For this reason, there are no prognostic factors that expect successful results with PRP treatment. However, this result could be due to a small number of patients and further studies with a larger number of subjects are needed to confirm this finding.

The efficacy and safety of autologous PRP have been reported in many fields of medicine, but few clinical trials exist to determine the role of PRP (18). The molecular mechanisms of PRP therapy in endometrial proliferation are currently not well understood. We hypothesize that intrauterine infusion of PRP helps expand the thin endometrium and improve the pregnancy rates. PRP is a relatively new treatment applied to improve endometrial thickness in women with a thin endometrium. It appears that PRP is safe due to the autologous nature derived from the patient's own blood.

Chang et al. reported the efficacy of intrauterine PRP infusion for endometrial growth in women with thin endometrium for the first time. Five patients with a history of thin endometrium (on the day of hCG administration) were recruited into the study. PRP was infused into the uterine cavity on the tenth day of the endometrial preparation cycle. If the endometrial thickness did not increase during the next 72 hours, the PRP infusion was infused 1 to 2 times in each cycle. When the thickness of the endometrium reached >7 mm, embryos were transferred. Successful endometrial expansion and pregnancy were observed in all patients after PRP infusion (12).

The LBR was noted in two previous studies (14,15). The first study reported 26.3% live births after PRP treatment, and the second study's live birth rate was 38.2%. The variation in LBR between the previous and current studies may be due to the difference in patient characteristics.

In this study the results of the PRP treatment cycle were compared with the reproductive outcomes of a patient group without the need for PRP treatment. Age, BMI, endometrial thickness, number of embryos transferred, number of good quality embryos transferred, implantation rate, clinical pregnancy rate and live births were not significantly higher in the treatment cycle with PRP compared to cycles without treatment, however, if there was a statistically significant difference in the proportion of the autologous and heterologous cycles when comparing both groups. A higher response was noted on the number of cycles with oocyte donation in the treatment group, which could represent alone a positive effect on our results not only due to in endometrial thickness increase, but also to a hypothetical grow in the proportion of euploid

embryos transferred, considering that a greater proportion of these are derived from younger donor ovules. A shortcoming of this study was the small group of patients, and insufficient information on the platelet concentration in each infused plasma preparation, however it is known that autologous blood plasma that has been enriched for platelets to approximately 4- 5 times more than circulating blood is able to upregulate Leukemia inhibitory factor (LIF) expression in endometrial stromal cells (19), and upregulating LIF expression could improve endometrial receptivity. It is also suggested that PRP may exert some impact in enhancing trophoblast placentation. PRP can stimulate proliferation and regeneration with many growth factors and cytokines, including Platelet derived growth factor (PDGF), Transforming growth factor (TGF), vascular endothelial growth factor (VEGF), Epidermal growth factor (EGF), fibroblast growth factor (FGF), insulin-like growth factor I, II (IGF I, IGF II), interleukin 8 (IL8) and connective tissue growth factor (CTGF).

Endometrial receptivity is controlled by dynamic and precise molecular and cellular cytokine events, transcription factors, and genes. Currently, PRP infusion is progressively used in various fields of medicine such as nerve injury, osteoarthritis, chronic tendinitis, bone repair and regeneration, heart muscle, alopecia, plastic surgery, and oral surgery, but there is limited experience in obstetrics and gynecology. For the first time, Chang reported the efficacy of intrauterine PRP infusion for endometrial growth in women with thin endometrium. In that trial, PRP was infused in 5 women with inadequate endometrium who had poor response to conventional therapy during FET cycle. Adequate endometrial thickness is a major factor for implantation and pregnancy, so women with persistent thin endometrium often do not undergo ET. Several methods have been described for the preparation of the endometrium, but there is still no gold standard. A number of investigators reported that granulocyte colony-stimulating factor (G-CSF) promotes endometrial growth, because this cytokine stimulates the differentiation and proliferation of granulocyte neutrophils and can induce endometrial proliferation and growth, and, therefore, improve pregnancy outcomes (21, 22). In agreement to this hypothesis, a local infusion of PRP containing various growth factors and cytokines can improve endometrial growth and receptivity.

CONCLUSIONS

The present study was conducted to determine the effects of autologous PRP treatment on the thin endometrium of women in preparation por ET, and the results obtained represent a noteworthy improvement considering the history of the patients. Even though more studies on the molecular basis of this PRP treatment are needed to reveal the exact mechanism of action and obtain more solid evidence on its beneficial effect in patients with thin endometrium. For this reason, we suggest additional clinical trials should be conducted in this context. Autologous PRP treatment for endometrial priming is a safe procedure, with minimal risks of infectious disease or immunological reactions since it is made from autologous blood samples.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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