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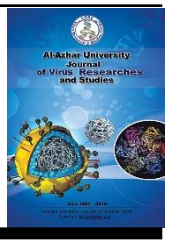

Effect of Autologous Platelet-Rich Plasma Treatment on Refractory Thin Endometrium during the Frozen Embryo Transfer Cycle

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Abstract

Platelet-rich plasma nowadays has been widely applied in different clinical scenarios, such as orthopedics, ophthalmology and wound healing to improve the tissue regeneration. However, little is known regarding the application of PRP in the treatment of thin endometrium. This study aimed to evaluate the Platelet-rich plasma (PRP) as an adjuvant therapy before embryo transfer in infertile women representing with refractory thin endometrium (<7 mm) during the frozen embryo transfer cycle. The present study was a randomized clinical trial prospective interventional study was conducted, involved 50 cases. Intrauterine autologous Platelet-rich plasma (PRP) administration was performed at the estrogen-primed FET cycle) . The EMT of 31 patients (62%) out of 50 still <7 mm after 1st inject and need other PRP inject. The EMT of 21 patients out of 31 still <7 mm after 2nd inject and need other PRP inject. The EMT of 15 patients out of 21 still <7 mm after 3rd inject and need other PRP inject. The EMT of 35 patients out of 50 (70%) reach more than 7 mm after PRP inject which is statistically highly significant increase in EMT after each PRP injection. Intra uterine PRP is effective in increasing endometrial thickness in reapted implantation failure in FET cycles due to refractory thin endometrium. Intra uterine PRP is easy, safe (being an autologous resource) and with very low-cost method.

Keywords: Platelet Rich Plasma, Endometrial Thickness, Implantation, Pregnancy, Infertile, Embryo.

1. Introduction

Endometrium is the tissue that lines the inner layer of the uterus and is essential for reproduction because embryo implantation takes place here [1].

An endometrium with thickness below 7mm is assumed as non-optimal for embryo implantation and is associated with a low pregnancy rate [2].

Females who have thin uterine lining can have some symptoms, as (infertility, abnormal menstrual cycle in the form of Painful periods and reduced menstrual bleeding) Many times women with thin endometrium can be without any symptoms and detected on ultrasound [3].

The causes of thin endometrium may be due to low oestrogen**.** In this case medication with oestradiol is prescribed, and its response monitored, Inadequate blood flow as in women who have

a sedentary lifestyle, presence of fibroids and polyps, poor health of endometrial tissue e.g. (any kind of bacterial infection like endometrial TB, sexually transmitted diseases and pelvic inflammatory diseases), Asherman Syndrome, birth control pills and Clomid also constrict the blood vessels, thus reducing blood flow to the endometrial lining [4].

For a successful pregnancy, a thin endometrial lining should be properly evaluated and treated accordingly to make it thick and nourishing so that it can accept an embryo. Some of the thin uterine lining treatment methods are: (Oestrogen therapy) oestrogen can be given orally or as a suppository gel, as it stimulates the division of cells in the endometrial lining so that it becomes thick and the fertilized egg can go and implant easily, human Menopausal Gonadotropin (hMG), Granulocyte Colony Stimulating Factor (G-CSF), Hysteroscopy for intrauterine adhesions, natural treatment e.g.(Vit E and L-arginine and regular exercise) but with poor response [5].

Platelet-rich plasma (PRP) is defined as aportion of the plasma fraction of autologous blood having a platelet concentration above baseline. Platelet-rich plasma nowadays has been widely applied in different clinical scenarios, such as orthopedics, ophthalmology and wound healing to improve the tissue regeneration. However, little is known regarding the application of PRP in the treatment of thin endometrium [6].

2. Patients and Methods

The present study is a randomized clinical prospective interventional study that was carried out at Obstetrics and Gynecology department at Al-Madina private Hospital during the period from January 2020 to June 2021.

The present study enrolled 50 patients who had thin endometrium<7mm during the frozen embryo transfer cycle. Intrauterine autologous Platelet-rich plasma (PRP) administration was performed at the estrogen-primed FET cycle. PRP started on day10 and repeated every 3-day s intervals if endometrial thickness still less than 7mm by ultrasound examination.

2.1 Inclusion Criteria

Informed consent form signed, age 20-40 year sold, thin endometrium less than 7mm on the end of estrogen priming day in frozen embryo transfer cycle, body mass index less than 30 and two or more failed IVF cycles due to thin endometrium and more or two cycles of previous therapy for increasing the endometrial thickness, such as, high dose estradiol valerate, transvaginal sildenafil administration, or pentoxifylline combination with vitamin E.

2.2 Exclusion Criteria

Hematologic disorders, hemoglobin level<9g dl, platelet count <100,00ml, autoimmune disease e.g., Rheumatoid Arthritis, chromosomal abnormality in the patient and uncontrolled endocrine or other medical condition such as Diabetes, prolactinoma or thyroid diseases.

2.3. Intervention

All patients registered along with written consent. Complete history taken age, parity, history of infertility, or history of failed two or more IVF cycles. Clinical examination (general, abdominal and local), laboratory investigation as (CBC, T3, T4, E2, progesterone, serum prolactin, coagulation profile and fasting and postprandial blood sugar) done. Before PRP administration, Ultrasonography done to measure the EMT on MCD 2& on day 10 and on each PRP administration day. The patients started to take a daily dose of 6 mg of estradiol valerate from menstrual cycle day (MCD) 2 to prepare the endometrium till the day10. Intrauterine autologous Platelet-rich plasma (PRP) administration performed at the estrogen-

primed FET cycle. On each Platelet-rich plasma (PRP) administration day, 18 mL of venous blood drawn from the patients using2 0 mL syringes, the blood was immediately put in a large sized sodium citrate tube. VACUETTE18 mL tube, anticoagulant solution. The blood samples then moved into an aseptic PRP centrifuge kit (HERMLEZ326K) and centrifuged at 1000 G for 3 min. The buffy coat and plasma just above the buffy coat collected, and 0.7–1.0 mL of PRP produced. The first autologous Platelet-rich plasma (PRP) infusion performed on MCD 10if end thickness still less than 7mm detected by vaginal ultrasound. PRP was repeated every 3-day intervals until the EMT reached 7 mm by vaginal ultrasound. PRP administered into the uterine cavity using an embryo transfer catheter within 1 h from completion of PRP preparation. The syringe containing the Platelet-rich plasma (PRP) connected to ET catheter and the Platelet-rich plasma (PRP) was infused Then the air bubble was confirmed in ultrasonography confirmed in ultrasonography. U/ S repeated at the end of 3rd PRP to measure the end thickness.

Thereafter, the patients prescribed with second-generation cephalosporin for 2 days as prophylaxis for infection (1g ceftriaxone every 24hour). The maximum number of autologous infusions limited to three. U/ S repeated at the end of 3rd PRP to measure the end thickness.

2.4 Statistical analysis

Statistical analyses of data were carried out using SPSS version 23. Shapiro –Wilks test was used to test normal distribution of variables. Numerical data were expressed as mean ± standard deviation or median and range. Categorical data were summarized as percentages. The significance for the difference between groups was determined by using two-tailed Student's t test. Also, Qualitative variables were assessed by chi-squared χ2test. The probability (P) values of $\leq$0.05 were considered statistically significant.

3 .Results

Table.1 shows that no patients were detected aged 20 -25y were detected. However, the largest no. of patients (30) was above 35 y. Table.2 shows BMI distribution of the studied group. The mean ± S.D value was 23.17±3.114. The maximum no. of patients (30) was of normal BMI. Table.3 shows parity distribution of the studied group. It shows that 42 patients had primary infertility (84. 0%) and 8 (16. 0%) had secondary infertility. Table.4 shows the duration of infertility of studied group, and it was ranged between 1.5-10.5 years with a mean value of 5.62±2.655 years. Table.5 shows that EMT at day 10 is more than that at day 2 which is statistically highly significant (P<0.001) but still less than 7mm. Table.6 shows that there was highly statistically significant increase in EMT after each PRP injection (P value<0.001). Table.7 shows That the EMT of 31 patients (62%) out of 50 was still <7 mm after the 1st injection and need another PRP injection. The EMT of 21 patients out of 31 was still < 7 mm after the 2nd inject ion and need another PRP injection. The EMT of 15 patients out of 21 was still < 7 mm after the 3rd injection. SO, the EMT of 35 patients out of 50 (70%) succeed to reach more than 7 mm after PRP inject ion which is statistically highly significant P value<0.001. Table.8 shows the outcome of the studied group; 22 (44%) out of 35 pt underwent PRP had got pregnancy and 5(10.0%) of them had aborted in 1st trimester. 13 (46%) patients had no pregnancy.

Table (1): Distribution of studied sample regarding patient's age.

Age (years)	Number	Percent
Range	25-40	
Mean ± S.D.	35.80±4.431	
20 – 25	0	0
25 – 30	5	10.0
30-35	15	30.0
>35	30	60.0

Table (2): Distribution of studied sample regarding patient's Body Mass Index (BMI).

BMI (kg/m^2)	Number	Percent
Range	17.7-28.60	
Mean ± S.D.	23.17±3.114	
Underweight 23.17±3.114<18.5)	3	6.0
Normal (18.5 – 24.9)	30	60.0
Overweight (25.0 – 29.9)	17	34.0

Table (3): Distribution of studied sample according to patient's parity.

Parity	Number	Percent
0	42	84.0
1	3	6.0
2	5	10.0

Table (4): Distribution of studied sample according to patient's duration of infertility.

	Duration of infertility
Range	1.5-10.5
Median	5.7
Mean ± S.D.	5.62±2.655

Table (5): Endometrial thickness (EMT) mm by ultrasound examination before PRP injection among studied group. (50 pt).

	EMT (mm)		P value
	At day 2	At day 10	
Range	3.1-6.5	4.2 -6.2	<0.001*
Median	4.9	5.5	
Mean ± S.D.	4.84±0.846	5.30±1.144	

Table (6): Patient's Endometrial thickness (EMT) before each PRP injection.

Ultrasound at PRP injection	EMT			P value
	At 1st inject.	At 2nd inject.	At 3rd inject.	
N of pat.	**50**	**31**	**21**	**<0.001***
Range of EMT (mm)	**4.2-6.2**	**4.6-6.8**	**5.6-6.6**	
Mean ± S.D (mm)	**5.30±1.144**	**5.531±0.947**	**5.59±0.610**	

Table (7): Distribution of studied sample according to patient's EMT after each PRP injection.

	PRP injection						P value
	After 1st inject.		After 2nd inject.		After 3rd inject.		
	No.	%	No.	%	No.	%	
EMT <7mm	31	62.0	21	67.7	15	71.5	<0.001*
EMT ≥7mm	19	38.0	10	32.3	6	28.5	
Total	50		31		21		

Table (8): Distribution of studied sample according to patient's outcome.

Outcome (n=50)	Number	Percent
Pregnant	22	44.0
1st Trimester abortion	5	10.0

4. Discussion

The present study showed that the duration of infertility was ranged between 1.5-10.5 years with a mean value of 5.62±2.655 years (table 4). In accordance with the present results, [7] reported that the mean +_SD duration of infertility in the 20 women was 5.7 year's (±10. 11) .

However, in the study of Prasad [8], the mean +_SD duration of infertility was (19.02) years .14 women had primary infertility while 10 had secondary infertility. The current study showed that endometrial thickness (EMT) at day 10 before PRP injection and after estrogen preparation is more than that at day 2(this increase in endometrial thickness is still less than7mm). which is statistically highly significant (P<0.001). The Mean± S.D of EMT at day 2 was 4.84±0.846 mm and at day 10 was5.3 ±1.144. mm (table 5). There was highly statistically significant increase in EMT after each PRP injection. The mean± SD of EM T before the first, 2nd and 3rd injections were 5.30±1.144, 5. 31±0.947 &5.59±0.610 respectively (table 6). The EMT of 31 patients out of 50was still <7 mm after the 1st injection and need another PRP injection. The EMT of 21 patients out of 31 was still <7 mm after the 2nd injection and need another PRP injection. The EMT of 15 patients out of 21 was still <7 mm after 3rd injection. So, The EMT of 35 patients out of 50 succeeded to reach more than 7 mm after PRP inject ions which is statistically highly significant (P<0.001) (table7).

Finally, from all these results in the present study, it could be stated that the success rate of PRP injection was (70%) (35patient out of 50). 19 patient vs 31, 10 patients vs21

and6 patent vs 15 their EMT succeeded to reach more than 7mm after the first, second and third PRP injection s respectively which is statically highly significant (P<0.001). The outcome of this study according to occurrence of pregnancy was 22(62%) patients out of 35 who underwent PRP had got pregnant. However ,5 of them had aborted in the 1st trimester (table8). A study by Chang et al., [1] evaluated the role of autologous PRP in thin endometrium in five patients undergoing frozen embryo transfer cycles. The EMT increased at 48 to 72 h after PRP infusion in all the patients and reached >7 mm on the day of progesterone administration. All the five patients were pregnant. One of the patients had a missed miscarriage secondary to a chromosomally abnormal fetus, whereas the other four had viable intrauterine pregnancies, followed up till the first trimester.

The present results were supported by study of Tandulwadkar et al., [9] as they reported that the mean pre-PRP endometrial thickness (E MT) was 5 mm which significantly increased to 7.22 mm post-PRP. There was a significant increase in vascularity, seen by the number of vascular signals detected by Power Doppler, reaching the zones 3 and 4 of the endometrium they reported that the positive beta Human Chorionic Gonadotropin (hCG) rate was 60.93% and the clinical pregnancy rate was 45.31%. A total of 13 women are in the second trimester, 13 are in the first trimester with a healthy intrauterine pregnancy, one patient had an ectopic gestation, three had blighted ova, two had missed abortions, and two biochemical pregnancies.

The results of Zadehmodarres et al., [10] 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 thin endometrium. Molina et al., [4] demonstrated that endometrial thicknesses > 7mm was reported with the first use of PRP injection; and in all cases, endometrial thicknesses >9mm were evident after the second administration. The entire study group qualified for Embryo Transfer at the blastocyst stage. they had 73.7% of positive pregnancy tests, of which 26.3% yielded live births; 26.3% ongoing pregnancies; 10.5% biochemical pregnancies; 5.3% anembryonic pregnancies and 5.3% had fetal death (16 weeks).

Furthermore, **El Hamedi & Salem, 2019** demonstrated that the endometrial thickness increased at 48-72 h after PRP infusion in all the patients and reached >7 mm on the day of progesterone administration.

Also, Nazari et al., [10] showed that endometrial thickness increased at 48 hr after the first intervention in both groups. All participants needed second intervention due to an inadequate endometrial expansion. After second intervention, endometrial thickness was 7.21 ± 0.18 and 5.76 ± 0.97 mm in the PRP group and sham-catheter group, respectively. There was a significant difference between the two groups. ($p < 0.001$). Twelve patients were excluded for different reasons, and 60 included patients were randomly assigned to PRP or sham-catheter groups in a double-blind manner. The study revealed that embryo transfer was done for all patients in PRP group and just in six cases in the sham-catheter group. Chemical pregnancy was reported in twelve cases in the PRP group and two cases in the sham catheter group. Prasad [8] stated that the mean pre-treatment endometrial thickness was 4.68±0.96 mm, which significantly increased to 6.65±0.52 mm, post treatment ($P < 0.05$). Four patients out of 24 in their study could not achieve an optimal pattern of endometrium after treatment and embryo transfer was postponed. the positive beta human chorionic gonadotropin rate was 55%, and clinical pregnancy rate was 45% among them. The study of Kusumi et al., [11], after PRP administration, the mean (SD) endometrial thickness on the 14th day was significantly increased by 1.27 mm (P

< .001) and 0.72 mm (P = .001) on the basis of the unblinded and blinded measurements, respectively. This study demonstrated that of the 36 patients, 32 (88.9%) underwent FET. The clinical pregnancy rate was 15.6%. This is due to the decrease no. of patients enrolled in their study. A review article by Garcia-Velasco et al., [12] performed an extensive literature review on the management of refractory endometrium. The review included studies with conventional hormone preparations (estradiol Valerate), as well as various drugs including acetylsalicylic acid (aspirin), sildenafil, vitamin E, GnRH agonists, HCG, I arginine, pentoxifylline among others: as well as autologous preparations as growth factors, mainly G-SF, stem cells, PRP and bone marrow. Finally, they concluded that despite the vast array of resources available today, it is still not easy to provide a pragmatic evidence-based approach that guides the clinician on how to improve refractory endometrium. In this sense and regarding the results of their study, it is considered important to complement them with the formal design of test protocols, as well as comparative studies to establish the efficacy between different drugs and preparation, besides the inclusion of several years of data collection.

5. Conclusion

Intra uterine PRP is effective in increasing endometrial thickness in reapted implantation failure in FET cycles due to refractory thin endometrium. Intra uterine PRP is easy, safe (being an autologous resource) and with very low-cost method.

6. Recommendation

Thin refractory endometrium still remains one of the challenges. The use of PRP preparation and its effect on the endometrium seems to increase endometrial receptivity and a consequent increase in implantation and pregnancy rate. So, it could be recommended that PRP should be included in the different protocols for endometrial preparation in Assisted Reproductions Technique (ART) either in fresh or frozen embryo transfer.
Further studies are recommended in terms of the population size as well as the approach of comparative studies between drugs and autologous preparation. Ethical approval: Approval of Departmental and Ethical Committees were obtained from quality education assurance unit, Faculty of Medicine, Al-Azhar University Egypt.

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