

PRP in recurrent implantation failure, hope or hype? A prospective randomized controlled study

Original
Article

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ABSTRACT

Introduction: The endometrial function and endometrial receptivity have been accepted to be major limiting factors in the establishment of pregnancy. In spite of improved almost all aspects of IVF: ovarian stimulation, embryo culture and transfer, the pregnancy rates still not satisfactory. The bottleneck is the process of implantation. Recurrent implantation failure (RIF) is one of the nightmares in reproductive medicine and despite several strategies that have been described for management; there is no universal agreement yet. Recently, intrauterine infusion of platelet-rich plasma (PRP) is described to promote endometrial growth and receptivity, PRP has been investigated as a therapeutic approach for several medical disorders in dermatology and rheumatology, but its use in IVF is still limited.

Aim: To evaluate the effectiveness of intrauterine perfusion of autologous platelet-rich plasma in improvement of pregnancy rate in RIF patients.

Design: Prospective randomized controlled study.

Patients and Methods: After ethical committee approval was obtained, 150 infertile women with history of RIF gave their consent to be included in this study, with age below 40 yrs, body mass index (BMI) below 30 kg/m². They were divided into 2 comparable groups ; all participants underwent antagonist protocol. In the study group, intrauterine infusion of (PRP) was performed 48 hrs before blastocyst transfer, pregnancy tests were done 12 days after ET.

Results: Out of 75 participants in each group, 32 got pregnant (43%) in the study group, compared to 11 pregnant participants (15%) in control group.

Conclusion: According to our study, PRP significantly improved the pregnancy rate and may be a new hope in RIF patients.

Key Words: Implantation, IVF, platelet-rich plasma, recurrent implantation failure.

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INTRODUCTION

RIF greatly affects the likelihood of clinical pregnancy after IVF cycles. However, there is little information on its pathogenesis and no consensus among clinicians and researchers on its diagnostic criteria and treatment^[1].

It is a distressing condition for patients and frustrating for clinicians and scientists, it is estimated that approximately 10% of women seeking IVF treatment will experience this particular problem^[2].

Despite expanding experience in advanced reproductive technologies and great improvement in infertility treatment, implantation failure is one of the major challenges^[3].

The process of implantation involves two main

components, a healthy embryo that should have the potential to implant and a receptive endometrium that should enable implantation. The “cross-talk” between the embryo and the endometrium that finally leads to apposition, attachment and invasion of embryos is mandatory for successful implantation and subsequent normal placentation^[3].

Recurrent implantation failure is a nightmare for both the patients and the doctor. It needs a wide range of investigations and there is a debate about the lines of management. Several methods have been performed for RIF management but there is little consensus on the most effective one. Blastocyst transfer, preimplantation genetic screening (PGS), assisted hatching, co-culture system, sequential transfer, hysteroscopy, endometrial scratching, salpingectomy for tubal disease, extra number embryo

transfer, natural cycle, oocyte donation, intra-tubal ET, immune therapy and endometrial receptivity array (ERA) have been used but till now the pregnancy rate after ICSI cycle in RIF patients still unsatisfactory^[4].

Intrauterine infusion of platelet-rich plasma (PRP) is a new approach that has been suggested for the treatment of thin endometrium^[5]. PRP is blood plasma prepared from fresh whole blood that has been enriched with platelets. It is collected from peripheral veins and contains several growth factors such as vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), platelet derived growth factor (PDGF), transforming growth factor (TGF) and other cytokines that stimulate proliferation and growth. Recently, PRP has been used in several medical conditions in ophthalmology, orthopedics, surgery and wound healing but its efficacy in endometrial growth has not been fully elucidated^[6, 7, 8, 9, 10, 11, 12].

AIM OF THE STUDY

The study aimed to evaluate the effectiveness of intrauterine perfusion of autologous platelet-rich plasma in improvement of pregnancy rate in RIF patients.

MATERIALS AND METHODS

Study design

This was a prospective RCT (registered at clinical trials.gov with ID: NCT04058783) which conducted in Al-Baraka Fertility Hospital, Manama, Bahrain. After ethical committee, an approval was obtained, 150 infertile women with history of RIF gave their written consent to be included in this study. All were recruited from the outpatient clinic of Al-Baraka fertility hospital 150 women were included in this study who failed to conceive after 3 or more ET with high-quality embryos were assessed for eligibility to enter the study from July 2018 to March 2019.

The inclusion criteria were age below 40 yrs., body mass index (BMI) below 30 kg/m². The exclusion criteria were hematological and immunological disorders, hormonal disorders, chromosomal and genetic abnormalities and

uterine abnormalities (acquired or congenital) as confirmed by HSG and U/S to limit additional factors that may affect the results of the study.

Laboratory evaluation of hormonal, hematological and immunological disorders was done then divided into 2 comparable groups; all participants underwent antagonist protocol, oocytes retrieval, fertilization and embryo transfer; Good quality blastocysts (Grade A or B according to embryologic scoring) transferred for all of the participants.

In study group (75 patients), intrauterine infusion of PRP was done 48 hours before ET. PRP was prepared from autologous blood and it was made by using two steps centrifuge process. All Blastocyst transfers were performed under ultrasound guidance by one expert gynecologist with infertility fellowship. ET was performed according to American Society for Reproductive Medicine (ASRM) guidelines 2013 (Two or three embryos for each participant). On PRP infusion day, 17.5 ml of peripheral venous blood was drawn into a syringe that contains 2.5 ml of Acid Citrate and centrifuged immediately at 1200 rpm for 12 min to separate red blood cells, then plasma was centrifuged again at 3300 rpm for 7 min to obtain PRP that contained platelet 4-5 times more than peripheral blood. 0.5- 1 ml of PRP was infused into the uterine cavity with embryo transfer catheter (Wallace - Smiths, UK). On the other side, No PRP was done in control group. Pregnancy tests were done 12 days after ET.

Outcome assessment

Pregnancy was determined by positive serum β -HCG, 12 days after ET.

RESULTS

A total of 150 participants with RIF history were entered into this study. All of them were able to complete the study and their data were analyzed. Table 1 provides baseline characteristics summary.

Participants had a history of failed previous ET attempts between 3-5 times and their mean age was 29.6 \pm 3.7 years.

Table 1: Demographic data of studied patients.

Variables	Groups	Study (N = 75)	Control (N = 75)	T-test	
				T	P value
Age (years)	Mean	29.3	29.9	1.1	0.2
	\pm SD	3.5	3.9		
BMI	Mean	26.7	26.6	0.5	0.6
	\pm SD	1.1	1.08		

PRP IN RECURRENT IMPLANTATION FAILURE

Infertility duration (years)	Mean	6.6	6.2	0.6	0.5
	±SD	3.7	4.4		
Cause of infertility	♂ factor	53 (71%)	42 (56%)	6.8	0.07
	Tubal factor	11 (15%)	21 (28%)		
	PCO	2 (2%)	0 (0%)		
	Unexplained	9 (12%)	12 (16%)		
AMH (IU/ml)	Mean	1.5	1.3	0.7	0.4
	±SD	0.8	0.7		
No. of previous failed trials	Mean	4.4	4.2	0.6	0.6
	±SD	1.1	1.1		

Table 2: Both groups were comparable in the characteristics of IVF cycles as shown in this table

Variables	Groups	Study (N = 75)	Control (N = 75)	T-test	
				T	P value
HMG (Amp)	Mean	36.7	41.1	1.5	0.1
	±SD	15.1	19.3		
Duration of stimulation (Days)	Mean	12.7	12.4	0.7	0.4
	±SD	2.4	2.7		
End. thickness (mm)	Mean	10.8	10.7	0.3	0.7
	±SD	1.9	2.2		
Oocyte retrieved	Mean	8.9	8.2	2.8	0.3
	±SD	4.2	2.8		
Oocyte fertilized	Mean	4.2	3.6	1.8	0.06
	±SD	2.3	1.6		
No. of trans. embryos	Mean	2.5	2.3	0.8	0.4
	±SD	0.8	0.7		

Table 3: Comparison between studied groups (study and control) as regards pregnancy test

Variables	Groups	Study (N = 75)	Control (N = 75)	T-test	
				T	P value
Pregnancy test	Negative	43 (57%)	64 (85%)	14.4	< 0.001*
	Positive	32 (43%)	11 (15%)		

*: p -value < 0.001 is considered highly significant.

Out of 75 participants in each group, 32 got pregnant (43%) in the study group, compared to 11 pregnant participants (15%) in control group. This table shows

highly statistical significant difference (p -value < 0.001) between studied groups (Study and Control) as regard pregnancy

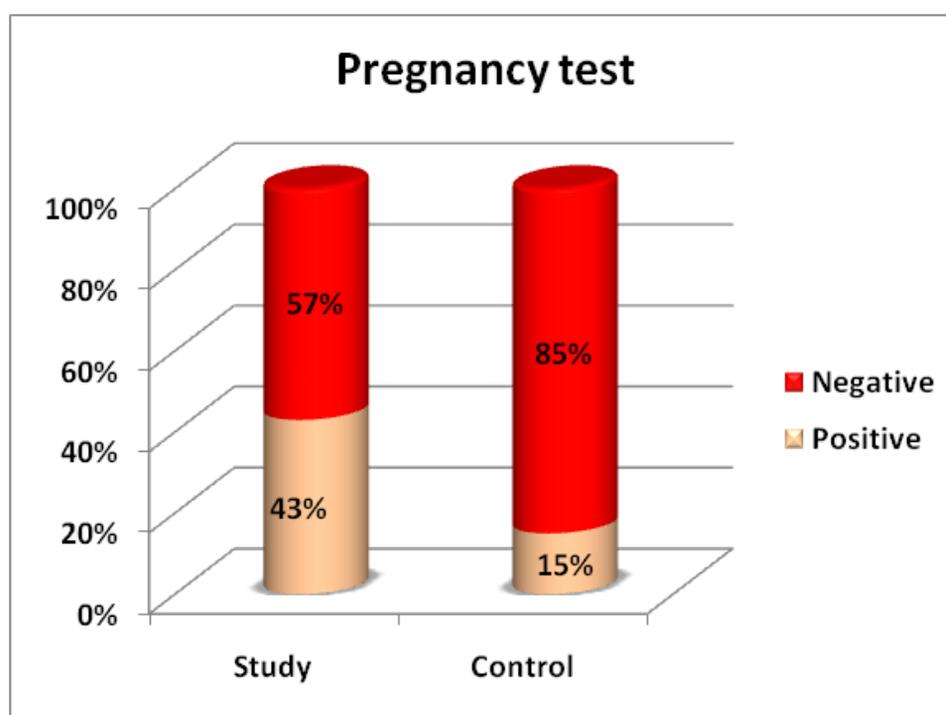


Fig. 1: Comparison between studied groups (Study and Control) as regards the pregnancy test.

DISCUSSION

Principal Findings

The result of our study revealed that endometrial perfusion of platelet-rich plasma (PRP) significantly improved the pregnancy rate and maybe a new hope in those patients with a history of recurrent implantation failure (RIF). Out of 75 participants in each group, 32 got pregnant (43%) in the study group after endometrial PRP infusion, compared to 11 pregnant participants (15%) in the control group.

Results of the study in the context of other observations

Still there is no universally accepted definition for RIF, but most fertility specialists agree that it refers to the failure to achieve a clinical pregnancy after transfer of at least four good-quality embryos in a minimum of three fresh or frozen cycles in a woman under the age of 40 years^[13, 14, 15, 16, 39].

This problem repeats itself and is frequently seen in most IVF units. Even after an IVF cycle in which everything

apparently goes well, after transfer of apparently good quality 3 embryos the pregnancy rates are around 50 %. In contrary, pregnancies could happen after transfer of poor quality single embryo.

From the clinical point of view, it is worthy to note that the term 'implantation failure' refers to two different types of situation, those in whom there has never been evidence of implantation (no detectable HCG production) and those who have evidence of implantation (detectable HCG production) but it did not proceed to beyond the formation of a gestational sac visible on ultrasonography. Implantation failure may be a consequence of embryo or endometrial factors^[17].

The endometrial function and endometrial receptivity have been accepted to be major limiting factors in the establishment of pregnancy. In spite of improved almost all aspects of IVF: ovarian stimulation, embryo culture and transfer, the pregnancy rates still not satisfactory^[18]. The bottleneck is the process of implantation.

Implantation is the process by which the embryo attaches to the uterine wall and first penetrates the epithelium and then the circulatory system of the mother to form the placenta. It's the most vital and the least understood part of reproduction.

The endometrium is normally a non-receptive environment for an embryo except during the window. Implantation window is a period during which the endometrium is optimally receptive to implanting blastocyst within the cycle days 20 and 24^[19].

The cross-talk between the endometrium and the developing embryo is mediated by many substances, including cytokines (IL1, IL6 and its product LIF), integrins, adhesion molecules, metallo-proteins, growth factors, prostaglandins and hormones such as hCG, all of which support the process of apposition, adhesion, and invasion^[20].

Unresponsive endometrium is difficult to treat and obviously contributes to implantation failure. Several approaches have been implemented to increase endometrial receptivity, and presumably prepare it for the "window of implantation". Treatment with high dose oral estrogen or vaginal estradiol application, intended to increase the estradiol level in the serum, as well as in the vicinity of the endometrium, has demonstrated only marginal success (12, 22). Similarly, treatment with low dose aspirin^[23] or vaginal sildenafil (24), which presumably increases blood flow to the uterus, consequently improving the response to estradiol, were rather disappointing^[25].

RIF may sometimes be associated with a thin endometrium (<7 mm) noted at the time of ultrasound examination on the day of HCG administration or embryo transfer. The observation suggests that the endometrium is not optimally responding to estrogenic stimulation. From

time to time, the underlying cause may not be obvious^[26].

Adequate endometrial thickness is the main factor for implantation and pregnancy. Women with persistent thin endometrium often do not undergo embryo transfer. Several methods have been described for endometrial preparation but there is not any definitive method yet. In recent years, intrauterine infusion of G-CSF has been studied but inconsistent results have been reported. Some researchers reported that G-CSF favors endometrial growth and pregnancy. G-CSF is a cytokine that stimulates neutrophilic granulocyte differentiation and proliferation, it may induce endometrium proliferation and growth, thus improve pregnancy outcome^[27, 28].

Sak and his colleagues investigated that expression of growth factors in the endometrium of women with RIF history is less than normal fertile women^[27, 29, 30, 31].

According to this hypothesis local infusion of PRP that contains several growth factors and cytokines may improve endometrial receptivity and implantation.

PRP is autologous blood plasma that has been enriched with platelets at about 4-5 times more than the circulating blood. PRP can stimulate proliferation and regeneration with a large amount of growth factors and cytokines, including PDGF, TGF, VEGF, EGF, fibroblast growth factor (FGF), insulin-like growth factor I, II (IGF I, II), interleukin 8 (IL-8) and connective tissue growth factor (CTGF). Currently, PRP infusion is being increasingly used in several fields in medicine such as nerve injury, osteoarthritis, chronic tendinitis, bone repair and regeneration, cardiac muscles, alopecia, plastic surgery and oral surgery, but there is limited experience in gynecology and obstetrics^[27, 32, 33, 34, 35].

Based on this assumption, local infusion of PRP that contains several growth factors and cytokines may add value. PRP is collected from autologous blood sample, so in comparison to G-CSF, PRP is more accessible and affordable^[27, 36, 37].

For the first time, Chang reported the efficacy of intrauterine infusion of PRP for endometrial growth in women with thin endometrium. In that trial, PRP was infused in 5 women with inadequate endometrium who had a poor response to conventional therapy during the FET cycle. The proper response to treatment was reported in all of them, and normal pregnancy was reported in 4 women^[5, 27].

Recently, Reghini and co-workers suggested the efficacy of PRP for the treatment of inflammatory response in chronic degenerative endometritis in mares. In this trial, 13 mares with endometrium classified as chronic degenerative endometritis and 8 mares with normal endometrial histology were selected to investigate the PRP therapy effect. The mares were inseminated with fresh semen in two consecutive cycles in a crossover

study design. Each mare served as its own control and the treatment was performed with intrauterine PRP infusion four hrs after artificial insemination. They concluded that PRP was effective in modulating the exacerbated uterine inflammatory response to semen in mares with chronic degenerative endometritis^[27, 38].

Just recently, the result of Leila Nazari, *et al.*, a study revealed the efficacy of PRP intrauterine infusion on implantation and pregnancy. In their trial, twenty women with a history of RIF who were candidates for frozen-thawed embryo transfer were recruited in that study. Intrauterine infusion of 0.5 ml of platelet-rich plasma that contained platelet 4-5 times more than peripheral blood sample was performed 48 hrs before blastocyst transfer and eighteen participants were pregnant with one early miscarriage and one molar pregnancy. Sixteen clinical pregnancies were recorded and their pregnancies are ongoing^[27].

STRENGTHS AND LIMITATIONS

The main strengths of our study include: 1) this is by far the first study discussing this topic in our country, as our hospital is the leader IVF center here in Bahrain. 2) The low risk of bias between the study and control group as both of them were statistically comparable. 3) We exclude those patients with hematological and immunological disorders, hormonal disorders, chromosomal and genetic abnormalities and uterine abnormalities (acquired or congenital) as confirmed by HSG and U/S to limit additional factors that may affect the results of the study. 4) All Blastocyst transfers were performed under ultrasound guidance by only one expert gynecologist with infertility fellowship. 5) The use of individual patient data for direct comparison between both groups. 6) The consistency between our results and those of the previous trials in the literature.

Some limitations have to be noticed, as we are living here in a small country so, the sample size may need to be increased in the further coming clinical trials, also at the time of embryo transfer we didn't select those embryos based on a genetic basis as we are usually not doing PGT as a routine; so we select the embryos phenotypically according to the embryo scoring.

CONCLUSION

The recurrent implantation failure is hard to be managed, and if all available treatments fail, then endometrial PRP can add value. The local infusion of PRP that contains several growth factors and cytokines may improve endometrial receptivity and implantation. PRP is collected from autologous blood sample, so in comparison to G-CSF, PRP is more accessible and affordable.

PRP can stimulate proliferation and regeneration with a large amount of growth factors and cytokines. Currently,

PRP infusion is being increasingly used in several fields in medicine but still not widely applied in infertility management.

In conclusion: The result of our study revealed that platelet-rich plasma significantly improved the pregnancy rate and maybe a new hope in RIF patients. Out of 75 participants in each group, 32 got pregnant (43%) in the study group after endometrial PRP infusion, compared to 11 pregnant participants (15%) in the control group.

CONFLICTS OF INTEREST

There are no conflicts of interest.

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