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Original Research Article

Effects of Intrauterine Infusion of Autologous platelet rich plasma on Endometrial Thickness, VEGF and Pregnancy Outcome in patients undergoing IUI

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Abstract

Current implantation is a well-orchestrated episode demanding the presence of a well embryo, a compassionate endometrium, suitable embryo endometrial cross-talk, and adequate maternal immune defense. In spite of developments in assisted reproductive technology, there are of no importance advances in the implantation and pregnancy rates. Intrauterine infusion of autologous platelet rich plasma might renew implantation rates through its paracrine properties by progression cytokines and growth factors which facilitate implantation. The aim of current study was to determine whether intrauterine distillation of autologous platelet rich plasma had a role on endometrial receptivity and pregnancy consequence of infertile Iraqi women. This is a randomized prospective study conducted in Higher Institute of Infertility diagnosis and Assisted Reproductive Techniques, AL-Nahrain University in the period from (July 2018 to July 2019). A overall of 43 patients who attend infertility clinic, where prospectively randomly dispersed into two groups. All of them were received Letrozol® tablet orally 2.5 mg twice daily 12 hours apart from day 2 for 5 days for one cycle. 20 women were control while 23 of them were given PRP by intrauterine infusion at day of hCG injection. IUI was done for entirely women in both groups 36-48 hour after confirming ovulation. Blood sample was collected from both groups at the day of IUI for valuation of vascular endothelial growth factor and an ultrasound was done at day of hCG injection and day of IUI for assessment of endometrial thickness. The mean endometrial thickness in PRP group at day of IUI was significantly thicker than that of control group land the difference in percentage change of endometrial thickness between PRP group and controls significantly higher in PRP group. Regarding the VEGF, it was higher than that in controls however. The Pregnancy rate in PRP group was significantly higher than of controls. VEGF was compared across the results of pregnancy tests in both studied group. There was no significant difference had been found neither between nor within groups. Autologous platelet rich plasma intrauterine infusion was safe, well-tolerated and give rise to in significant endometrial thickness expansion after PRP infusion, advance endometrial receptivity by increasing growth factors one of them in current study was VEGF and increase pregnancy rate in women with infertility underwent ovulation induction and intrauterine insemination.

Keywords: Human Chorionic Gonadotropin, Intrauterine insemination, Platelet rich plasms, vascular endothelial growth factor and in vitro fertilization

INTRODUCTION

Infertility is defined as failure to achieve pregnancy within 12 months of unprotected intercourse in women younger than 35 years or within 6 months in women older than 35 years (Pfeifer et al., 2015). Prosperous implantation accommodates receptive endometrium, profuse embryo quality with perfect embryo transfer technique (Eftekhar et al., 2016). Endometrial receptivity is regimented by dynamic and accurate molecular and cellular events of cytokines, homeobox transcription factors (regulate the expression of targeted genes and direct the formation of frequent body structures through early embryonic development and genes (Zhang et al., 2013). Usually,

the intrauterine insemination (IUI) is considered first line of dealing for couples suffering from different causes for infertility, encompassing cervical Infertility, ovulation dysfunction with minor to moderate male factors and for unexplained infertility (Fauque et al., 2014). It is cost effective, less aggressive, and a temporary phase previous to the application of stylish Assisted Reproductive technologies (ART) like in vitro fertilization (IVF) (Koli et al., 2013). Letrozole is a reversible selective third-generation aromatase inhibitor and has potential role to be used for ovulation induction with the suggestion of endometrial sparing effect. Letrozole improves ovulation by inhibition of the conversion of androgens to estrogens with making an estrogen-deficient state 2timulant the central drop of negative feedback by which the CC acts (Requena et al., 2006).

The platelet rich plasma (PRP) is defined as the plasma portion of autologous blood with a platelets concentration four to five times above normal (Lee et al., 2013). The platelets solid granules contain frequent growth factors placed and unrestricted by platelets, there are the insulin-like growth factor, the epidermal growth factor (EGF), the vascular endothelia growth factor (VEGF), platelet derived growth factor, the basic fibroblast growth factor, the transforming growth factor b-I and the hepatocyte growth factor (Cole et al., 2010).

Endometrial thickness is identified for instance the highest expanse between echogenic interfaces for myometrium and endometrium and was measured according to a level pass the central longitudinal axis of the uterus (Ting et al., 2015). The endometrial thickness is reliant on a number of influences including reproductive age, menstrual cycle phase, concentration of ovarian hormone progesterone and estrogen and endometrial hormone receptor density (Paulson, 2011). The thickened endometrium carry a spot for attachment, and it is the source of nourishment for an implanting embryo for the period of its first few weeks until advance of placenta (Senturk and Erel, 2008).

Vascular endothelial growth factor (VEGF) family is well categorized .VEGFs are homodimeric, glycoprotein growth factors that are particular to endothelial cells (Ferrara et al., 2003). They standardize angiogenesis and vascular permeability, mainly during embryogenesis, skeleton growth and reproductive functions. They also play important roles in hematopoiesis (Ferrara, 2004). The VEGF is necessary for prompt spurt of angiogenesis which happens throughout post menstrual and additional role in endometrial reepithelialization (Birbrair et al., 2015).

SUBJECTS, MATERIALS AND METHODS

A randomized prospective study conducted in Higher Institute of Infertility diagnosis and Assisted Reproductive Techniques / AL-Nahrain University in the period from (July 2018 to July 20l9). 43 women were involved in this study and was approved by the Local medical ethical committee of High Institute for Infertility Diagnosis Assisted Reproductive Technologies. The patients had the following criteria, Female aged 18-40 years, normal seminal fluid analysis, negative virology screen whereas the exclusion criteria were Female age <18and >40, Hb <11g /dL and platelet <150.000 /mm3 , female on anticoagulant treatment or take NSAIDS in 10 days before procedure and any significant comorbidity and psychiatric disorder. Forty three women with history of primary or secondary infertility were categorized in to two groups; the PRP group twenty three women received (Letrozole® tablet, Accord, UK.) orally 2.5-5 mg twice daily 12 hours separately from day 2 for 5 days.PRP was done at day of trigger and finding of a mature follicle with ≥17 mm diameter by vaginal ultrasound and endometrial thickness was measured. IUI was done after 36-48 hour after receiving hCG injection with approving ovulation and endometrial thickness assessment was done. The non PRP groups were 20 women and received the same protocol of induction. Ultrasound was done for assessment of endometrial thickness at day of trigger upon recognition ofa mature follicle with ≥17 mm diameter and at day of IUI. IUI was done after 36-48 hour after receiving hCG injection and confirming ovulation. Blood sample was taken to measure vascular endothelial growth factor on day of IUI for both PRP group and non PRP group.

PRP preparation

The blood was collected in special sterile vacationer PRP tubes containing an anticoagulant Na citrate 3.8%, which centrifugally splits red blood cells from plasma that comprises 'buffy coat' (platelets and white blood cells). The PRP tube was centrifuged at 1100 rpm for 6 min at room temperature. The plasma was quietly aspirated from the tube into a syringe and transferred to a second tube then centrifuged again at 3000 rpm for 10 min at room temperature, thus gaining a two-part plasma: the upper part, consisting of platelet-poor plasma, and the lower part, consisting of PRP. The platelet-poor plasma was first slightly aspirated to avoid its mixing up with the platelet concentrate and lower one third mixed with platelet concentrate (Nancy and Fatma, 2014).

RESULTS

Both two groups, patient characteristics are shown in Table (1, 2, 3). There was no significant differences between the two groups were detected in duration, type of infertility age, body mass index (BMI) and baseline hormonal levels.

No statistically significant differences had been found

Table 1. Distribution of the types and duration of infertility of the studied groups

Variable		Group				P. value
		PRP (N = 23)		Control (N = 20)		
		No.	%	No.	%	
Type of infertility	Primary	16	69.6	15	75.0	0.692 ns
	Secondary	7	30.4	5	25.0	
Duration of infertility (years)	*mean ± SD**	4.7 ± 2.2		4.5 ± 2.8		0.780 ns
	Range	1 – 10		1 - 11		
SD: standard deviation, ns: difference is not significant*						

Table 2. Agel and IBMI distribution of the studied groups

Variable		Group				P. value
		PRP (N = 23)		Control (N = 20)		
		No.	%	No.	%	
Age (year)	≤ 20	6	26.1	5	25.0	0.488 ns
	21 - 30	14	60.9	12	60.0	
	> 30	3	13.0	3	15.0	
Mean age ± SD* (year)		25.5 ± 4.7		26.2 ± 5.2		0.455 ns
BMI category	Normal	5	21.7	4	20.0	0.922 ns
	Overweight	11	47.8	10	50.0	
	Obese	7	30.4	6	30.0	
Mean BMI ± SD (kg/m^2)		27.7 ± 4.1		27.9 ± 3.5		0.891 ns

Table 3. Comparisons lof hormonal levels betweenlthel studied groups

Variable	Group		P. value
	PRP (N = 23)	Control (N = 20)	
	Mean ± SD	Mean ± SD	
FSH (mIU/ml)	7.0 ± 1.6	7.2 ± 2.2	0.712 ns
LH (mIU/ml)	5.4 ± 2.3	5.2 ± 1.3	0.760 ns
Prolactin (ng/ml)	15.8 ± 6.4	18.1 = 6.9	0.364 ns
TSH (mIU/ml)	1.9 ± 0.80	2.0 ± 0.72	0.658 ns

Table 4. Comparisons of Mean Number of Follicles, day of hCG and day of IUI between the studied groups

Variable	Group		P. value
	PRP (N = 23)	Control (N = 20)	
	Mean ± SD	Mean ± SD	
No. of follicles	1.6 ± 0.6	1.5 ± 0.6	0.723 ns
Day of hCG Injection	12 ± 2	13 ± 1	0.322 ns
Day of IUI	14 ± 2	15.1 ± 1.0	0.267 ns

between both studied groups in the number of follicles, day of hCG or day of IUI, (P>0.05 not significant), as shown in (Table 4).

In concerning the VEGF, the mean level was 258.1 ± 184.2 in PRP group and it was higher than that in controls (206.3 ± 119.04), however, the difference did not reach the statistical significance as shown in (Figure 1).

In concerning Figure (2), Figure (3) and Table (5), reviews the comparisons of the endometrial thickness (ET) at day of hCG and at day of IUI in two ways of

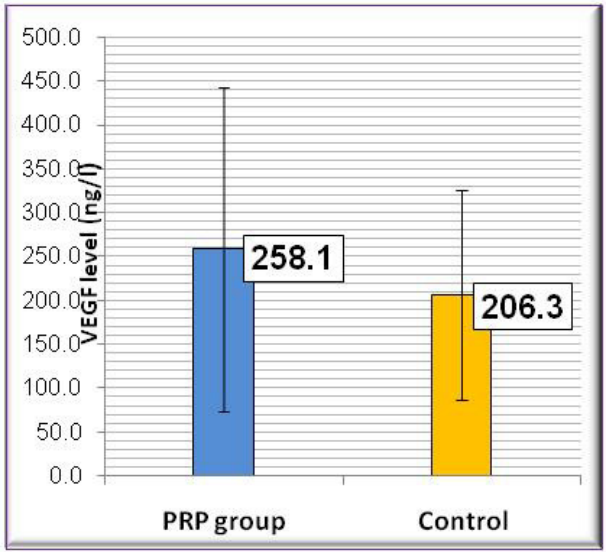

Figure 1. Bar chart showing the Comparisons of means levels of VEGF of the studied groups

Table 5. Comparisons of mean Endometrial Thickness at day of hCG and day of IUI within and between the studied groups

Endometrial Thickness (ET)	Group				P. value between groups
	PRP (N = 23)		Control (N = 20)		
	Mean	SD	Mean	SD	
ET at day of hCG (mm)	6.7	1.7	7.1	1.2	0.395 ns
ET at day of IUI (mm)	9.9	1.4	8.4	0.9	0.001 sig
Mean difference (mm)	3.2	1.6	1.3	0.5	0.001 sig
Percentage change	47.8%	8.4%	18.3%	1.7%	0.001 sig
P. value within groups	< 0.001 sig		< 0.001 sig		

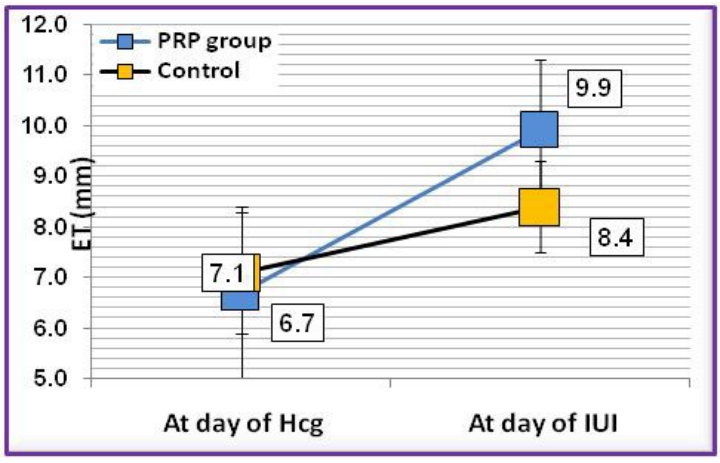

Figure 2. Box-plot chart showing the change in the ET values of both studied groups

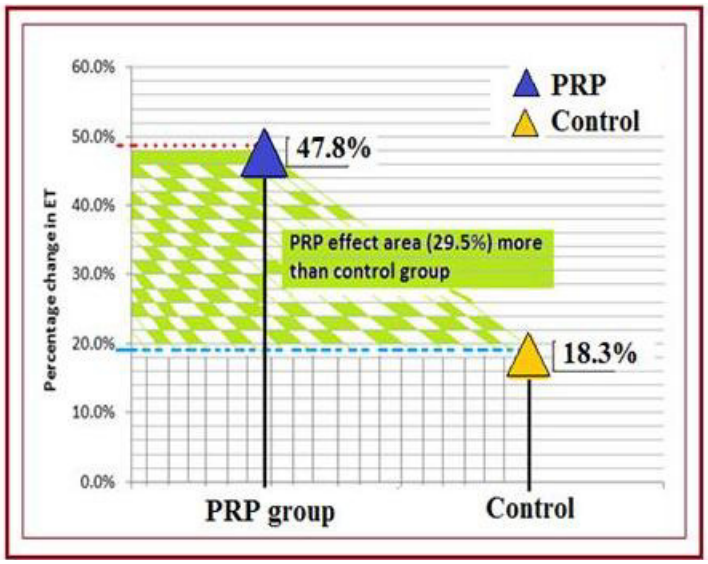

Figure 3. Comparison of percentage change in ET after treatment with PRP showing the area of effect of PRP

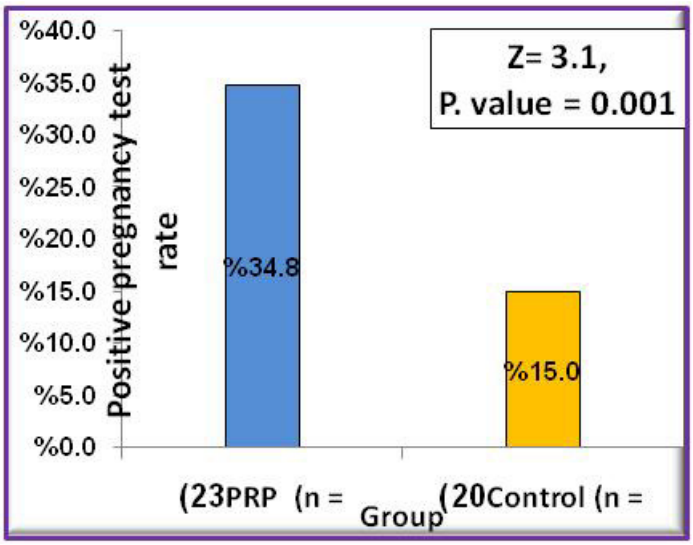

Figure 4. Assessment of Positive pregnancy test rates between both groups using Z statistics.

Table 6. Contrast of mean VEGF according to results of pregnancy test in both studied groups

Group	Pregnancy test	No. of patients	VEGF		P. value within group positive vs. negative
			Mean	SD	
PRP (N = 23)	Positive	8	**266.38**	203.27	0.765
	Negative	15	**242.57**	153.37	
Control (N = 20)	Positive	3	**250.36**	192.04	0.689
	Negative	17	**198.36**	108.57	
P. value between groups for positive pregnancy test			0.782 ns		
P. value between groups for negative pregnancy test			0.239 ns		

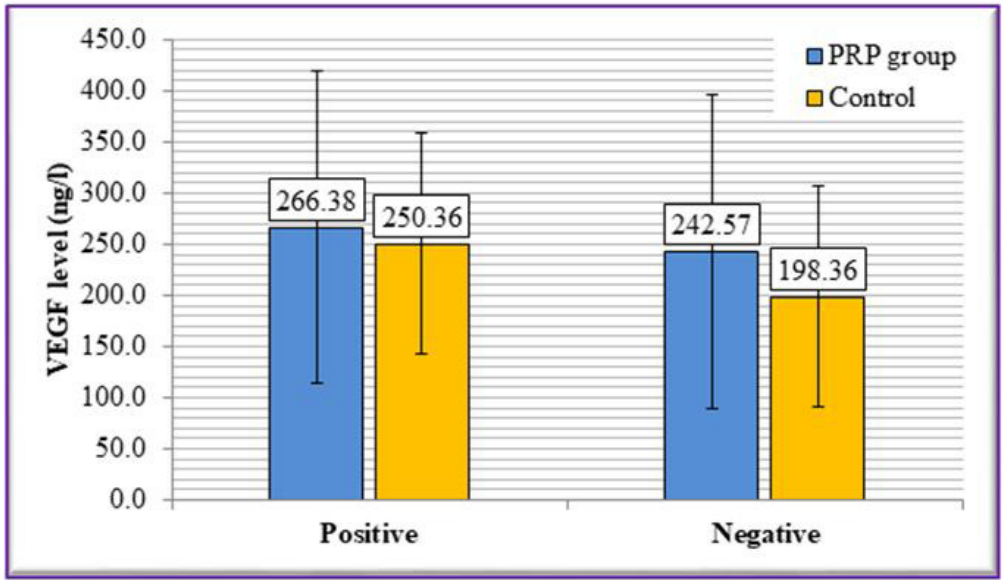

Figure 5. Comparison of mean VEGF according to results of pregnancy tests in both studied groups

comparisons; between groups (PRPvs. controls) and within each group (ET at day of hCG against ET at day of IUI). It was committed that at day of hCG the mean ET in PRP group and in control group with no statistically significant difference. At day of IUI the mean ET in PRP group was significantly higher than that of control group (P. value = 0.001). The comparison within group revealed a statistically significant difference between mean ET at day of IUI and that at day of hCG in both studied groups. In the PRP group the mean difference at day of IUI with a percentage change significantly higher than its value at day of hCG, (P< 0.001). In control group there was also a significant change of in ET with a percentage change at day of IUI than that at day of hCG, (P<0.001), however, the mean difference and percentage change of ET in PRP group was significantly greater than that of controls, in both comparisons (P = 0.001).

Regarding the pregnancy rate in PRP group was 8/23 (34.8%) compared to 3/20 (15%) in control group, when the positive pregnancy test rates in both groups compared using the Z statistics, it revealed that the 34.8% positive pregnancy test rate in PRP group was significantly higher than the 15% of controls (Z=3.1, P= 0.001), (Figure 4).

Additionally, the VEGF was compared across the results of pregnancy tests in both studied group. For VEGF was higher in pregnant group although no significant difference had been found neither between nor within groups, (P>0.05) (Table 6 and Figure 5).

DISCUSSION

Recurrent implantation failure etiology can be convened into 3 chief categories: embryonic defects declined endometrial receptivity and unsynchronized dialogue between embryonic and maternal tissues (Fatemi and Popovic-Todorovic, 2013). Platelet-rich plasma (PRP), include a platelet concentration of at minimum 1 000 000 platelets/L in 5 mL of plasma, having a 3-to-5-fold upsurge in growth factors concentrations, which is associated with the increase of healing (Berner et al., 2012). These factors signify signaling molecules which bind to specific receptors on target cells surfaces, supporting cell differentiation, proliferation and maturation and they are commonly concomitant with positive cell signaling (Garcia-Velasco et al., 2016).

The current study revealed that endometrial thickness at day of IUI was significantly higher in PRP group compared with control group as shown in (Table 5), (Figure2). This is agreed by study appraised the role of autologous PRP on thin endometrium in five patients undergoing frozen embryo transfer cycles. The endometrial thickness amplified at 48 to 72 h after PRP infusion in all the patients and extended>7 mm on day of progesterone administration (Chang et al., 2015). The present study presented that the mean level of VEGF in the PRP group was higher than that of controls, however the difference did not reach the statistical significance, and this is was shown in (Figure 1) . This is agreed by a study considered a PRP based framework in combination with mesenchymal stem cells and they found VEGF secretion from PRP contributed to the endothelial cells migration, as a result leading to osteogenesis and angiogenesis (El Backly et al., 2013).

In the present study the pregnancy rate in PRP group was 8/23 (34.8%) significantly higher compared to 3/20 (15%) in control group as shown in (Figure 4). This is agreed with studies which found that intrauterine PRP

infusion rise pregnancy rate in ICSI frozen cycles. A study achieved an experimental trial using PRP to increase quality of endometrial and implantation rates in patients have refractory endometrium and investigated that growth factors expression in the endometrium of women with RIF is fewer than normal fertile women (Molina et al., 2018). According to this hypothesis, local infusion of PRP that encompasses several growth factors and cytokines may intensificate endometrial receptivity and implantation (Hiroshi, 2006).

One of these growth factores that included in the present study is the VEGF, it is compared across the results of pregnancy tests in both studied group. For VEGF, it is higher in pregnant women than non- pregnant ones in both groups although there is no significant difference between or within groups seen (Table 6 and Figure 5), it could be due to small sample size. Furthermore, higher serum VEGF concentrations in an IVF cycle on the day of oocyte retrieval were prognostic of subsequent pregnancy (Dorn et al., 2003). VEGF is also formed by the invading blastocyst and prompts vasodilatation and angiogenesis (Daniel et al., 1999).

CONCLUSIONS

1. The use of PRP is considered safe because it is autologous and is derived from patient's own blood.
2. PRP intrauterine infusion could be an effective alternative treatment for patients with thin endometrium, since PRP significantly improve endometrial thickness.
3. Endometrial receptivity markers increase after use of PRP, though VEGF increase, it is not significantly.
4. Pregnancy rate in women with infertility undergoing IUI was improved significantly after using of intrauterine infusion of PRP at day of ovulation trigger.

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