How Does Platelet-Rich Plasma Injection in Ovaries of Poor Responders Affect the Retrieved Oocytes, and Anti Mullerian Hormone: A Clinical Trial

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Abstract
Objective: Platelet Rich Plasma (PRP) is proposed to have important role in cell division and proliferation, angiogenesis and health. This study evaluates the effect of a single injection of autologous PRP on ovarian response markers in women with poor ovarian response (POR).
Materials and methods: This non-randomized clinical trial was conducted between August 2020 and September 2021. Fifty six women with Bologna criteria for POR willingly chose to participate in one of the following groups: PRP for one cycle in the time of oocyte pickup (OPU) (intervention group, n= 34) or control group (n=22).The primary outcomes were: number and quality of oocytes in coming 2 cycles of ICSI, and Anti Mullerian Hormone (AMH) level two months after PRP injection. The secondary outcomes were the number and quality of embryos and chemical pregnancy rate after embryo transfer.
Results: A total of 45 participants continued the study, of which 23 were in the intervention group and 22 in control group. There were no demographic differences between two groups. At a two cycle follow up, PRP group experienced a significant improvement in AMH level and there was no respective change in control group. In one year follow up the overall pregnancy rates were same in both groups (3% Vs. 0, p=.60), while there was no difference in cumulative number and quality of embryos.
Conclusion: PRP injection can improve ovarian reserve marker without adverse effects. Further evidence is required to evaluate the impact of PRP on assisted reproduction outcomes.

Keywords: Platelet-Rich Plasma; Anti-Mullerian Hormone; Ovarian Reserve; In Vitro Fertilization

Introduction

Poor ovarian response (POR) patients need gonadotropin to enable an adequate ovarian function response to the ovulation stimulation. POR is usually the result of a decrease in both ovarian reserves and in the number of oocytes obtained during the in vitro fertilization (IVF) procedure. These patients appear to have a decrease in clinical pregnancy and live birth rates (1).

POR is defined as one or a combination of factors including: over 40 years of age, increased follicle stimulating hormone (FSH) on day 2 to 4 of menstrual cycle, cancellation of the previous cycle due to insufficient ovarian response after ovarian stimulation or fewer than three follicles and less than one follicle after oocyte pick-up (OPU) (2). In these women, treatment cycles are often canceled due to poor treatment response, which imposes an emotional and financial burden on these women and their spouses (3).

Anti-Müllerian hormone (AMH) is a potential marker for predicting ovarian response prior to ART (4-6). This hormone can be used to assess fertility potential and ovarian response in women who undergo IVF because of the association between serum AMH levels and the number of primary antral follicles (7).

Treatment of women with POR remains as a challenge. The purpose of ovarian stimulation in IVF is to have several follicles suitable for embryonic development (8).

It has been reported that platelet-rich plasma (PRP) can be effective in proper endometrial growth and increase the chances of pregnancy in cases where uterine endometrial tissue does not grow properly during frozen embryo transfer cycles (9-12). Intra-ovarian injection of PRP has been used in patients that had severely reduced ovarian reserve and were at risk for premature menopause. However, more extensive research is needed (13).

Intraovarian growth factors play important roles in local regulation and modulation of follicular selection and its development (14-16).

Platelets can release several growth factors, especially PRP contain 3-5 times more autologous human platelets than the basal level (17). The basal level contains a variety of hormones, adhesive molecules, cytokines, chemokinase, coagulation factors, integrin and growth factors such as transforming growth factor-β (TGF-b1, TGF-B2), insulin-like growth factors 1 and 2 (IGF-1 and IGF-2), and vascular endothelial growth factor (VEGF), which is an epithelial growth factor and an epidermal growth factor (17, 18).

The results of recent animal and clinical studies have shown the beneficial effects of PRP on infertility through the regeneration mechanism. PRP is a new treatment; therefore, confirmation of its effectiveness requires more clinical and research evidences (19).

Although PRP is thought to be useful in delaying follicle atresia and oocyte destruction, there is no conclusive evidence. Therefore, in this study we aimed to evaluate the results of PRP intraovarian injection in women with POR.

Materials and methods

Study design: This non-randomized clinical trial was conducted in an infertility treatment clinic affiliated to Tehran University of Medical Sciences, Tehran, Iran, between February August 2020 and September 2021(*Registration ID in IRCT: IRCT20141217020351N11.*

This study enrolled women who were considered to have POR. Women who met the following inclusion criteria were included in the study: at least one previous unsuccessful embryo transfer cycle, The Bologna criteria for POR [aged > 40 years, history of poor response (<3 oocytes per cycle), AMH <1.1 ng/mL] (20) , body mass index (BMI) <30 kg/m², minimum sperm count of $1x10^7$per cc, and the presence of at least 3% sperm that had normal morphology.

Exclusion criteria consisted of immunological diseases; hematological diseases; chromosomal or genetic disorders; uterine disorders) ; hemoglobin level <11 g/dL; platelets <150 000/mm³; systemic use of corticosteroids within two weeks before the procedure, underlying diseases of infertile women such as severe anemia; renal failure ; respiratory tract infections; endometriosis; submucosal myoma; asherman syndrome; untreated hyperprolactinemia and pathology of the fallopian tubes (hydrosalpinx, etc.), endocrine diseases and non-contraindications for pregnancy; allergic reactions; or dissatisfaction of the patient to continue treatment.

A total of 56 IVF candidates enrolled in the study after they met the inclusion criteria and provided informed consent. The patients were according to their own wish and non-randomly divided into two groups of intervention (n=34) and control (n=22). Patients in the intervention group received PRP during OPU.

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The control group did not receive any intervention during OPU. Serum AMH levels were measured in all patients after two months. Statistical tests showed that the demographic variables had no statistically significant difference (Table 1). Women in both groups underwent an antagonist regimen that included treatment of ovarian stimulation from the second day of the menstrual cycle (Cinnal F ampule; 150 IU, S.C.), Cinnagen Co., Iran) plus HMG (ampules; 150 IU, IM,) (PD HoMoG, Pooyeshdaru Co, Iran).

When at least one follicle above 14 mm was visualized by vaginal ultrasonography, the patients were given subcutaneous injections of Cetrotide 250 mcg) (Merck Co, Germany). When the follicle size reached 17-18 mm, the patients also received HCG (10000 units) injections (PD Preg, Pooyeshdaru Co, Iran). After 36 hours, the oocyte was picked up when the patient was under anesthesia, and PRP was performed according to a standard protocol (11, 12, 21). Immediately after the oocytes puncture, PRP was injected into each ovary by non-surgical,

transvaginal ultrasound-guided by a specialist. Two months after PRP; the patient re-entered the IVF cycle. The oocyte quality and the resulting embryos were evaluated. The majority of embryos due to the fact that the patient is POR; were freezing (embryo banking).

Each patient underwent an ultrasound evaluation to determine the appropriateness of the endometrium (presence of a triple line pattern) for embryo transfer. The embryo transfer was performed 3-5 days after puncture. In cases where the endometrium was not suitable for embryo transfer or if the mother selected to freeze the embryos, the patient underwent IVF during the following menstrual cycle.

Ovarian platelet-rich plasma (PRP) injection: First, 35 ccs of blood was taken from the patient's peripheral vein and the platelet count was checked .The patient's blood was centrifuged twice to remove the red blood cells and the platelet count was rechecked, and 6 ccs of PRP is provided by a kit (RooyaGen Co; Iran. The method of preparing PRP is explained in detail in this article (21).

Table 1: Pre-treatment patient characteristics in two groups

	Intervention (n=34)	Control (n=22)	P-value
Age (years)* (mean±SD)	39.34±3.88	40.81±3.68	0.307
BMI* (mean±SD)	25.20±4.43	25.20±4.07	0.169
Infertility type (n, %)**			0.602
Primary	23 (67)	15 (68)	
Secondary	11 (32.5)	7 (32)	
Baseline FSH* (mean±SD)	12.21±12.68	15.54±16.36	0.551
Baseline LH* (mean±SD)	9.21±8.75	9.24±10.03	0.651
Baseline AMH (ng/ml)* (mean±SD)	0.40±0.76	0.62±0.49	0.003
Baseline total AFC (n)* (mean±SD)	4.14±1.79	4.09±1.79	0.912
IVF outcome (mean±SD)*			
Oocyte	1.04±1.29	1.04±0.78	0.571
Embryo	4.21±1.31	3.68±1.42	0.487
Oocyte quality (n, %)***			0.448
M2	18 (53)	14 (63.5)	
M1	5 (15)	3 (13.5)	
GV	1 (3)	2 (9)	
None	10 (29)	3 (14)	
Embryo quality (n, %)***			0.281
A	0	1 (4)	
AB	11 (32)	9 (41)	
B	2 (6)	3 (14)	
All of them	3 (9)	0	
None	18 (53)	9 (41)	

BMI: Body mass index; AMH: Anti-Müllerian hormone; FSH: Follicle stimulating hormone; LH: Luteinizing hormone; IVF: In vitro fertilization
AFC: Antral follicle count.
*Mann-Whitney test; **Fisher's exact test; ***Chi-square test
None; Failure to form of oocyte or embryo

To prevent loss of growth factors, PRP was extracted before clot formation. Immediately after the puncture, Two cc PRP was injected into each ovary by a fixed infertility fellowship under anesthesia at four points via a 35cm 17G Cook Double Lumen Aspiration Needle using the guide of transvaginal ultrasound (HS-2600, Honda Electronic Co., LTD, Japan, 12.5 MHz) .For the control group, the catheter was placed and subsequently removed without injecting any substance.

The oocytes were fertilized by sperm in the laboratory and the resultant embryos were transferred or frozen according to the patient's age and number of available embryos. AMH levels were measured with an Ultra-Sensitive AMH/MIS ELISA assay methods by a kit (Pishtazteb Co., Iran) before and two months after PRP in both groups. Following PRP treatment, all patients in both groups were monitored by ultrasound. After their upcoming menstrual cycle, natural IVF cycles were performed in the treatment group.

The primary outcome (dependent variable) was the number and quality of oocytes and AMH level, whereas the secondary outcomes were the number and quality of the embryos, and chemical pregnancy after the second IVF cycle (two months after PRP).

Evaluation of the number and quality of oocyte and embryos quality were based on the embryologist's diagnosis in the first two IVF cycles of after PRP in the same year (22). If a suitable embryo was formed, the patient underwent an embryo transfer cycle or FET [Frozen Embryo Transfer]. Two weeks after the transfer, serum beta hCG levels were measured and positive serum beta hCG were considered successful.

Ethical consideration: The Institutional Review Board and Ethics Committee of Tehran University of Medical Sciences approved this study. All women who planned to undergo fertility treatment (IVF/ICSI) provided written informed consent for study participation. All patients were completely informed about the clinical trial study and the method of stimulation during this research.

Statistical analysis: Statistical analyses were performed using SPSS software (version 20, SPSS, Inc., IL, USA). P-values <0.05 indicated statistical significance. Quantitative variables are described as mean±SD and percentage. The Chi-square test or Fisher's exact test was used to compare qualitative variables between the two groups.

The Kolmogorov-Smirnov test indicated that the distribution of all quantitative variables was not normal; therefore, the non-parametric Mann-Whitney U-test was used to compare quantitative variables between the two groups. Changes in some quantitative variables, such as the AMH level and number of oocytes after the intervention compared to pre-treatment, were compared as a quantitative consequence between the two groups.

Results

A total of 56 women were assessed for eligibility. There were 34 women in the intervention group in this comparative analysis. Of these, 11 patients declined to continue the study and follow-up. Therefore, 23 patients continued with PRP treatment and 22 patients received no intervention (Figure 1).
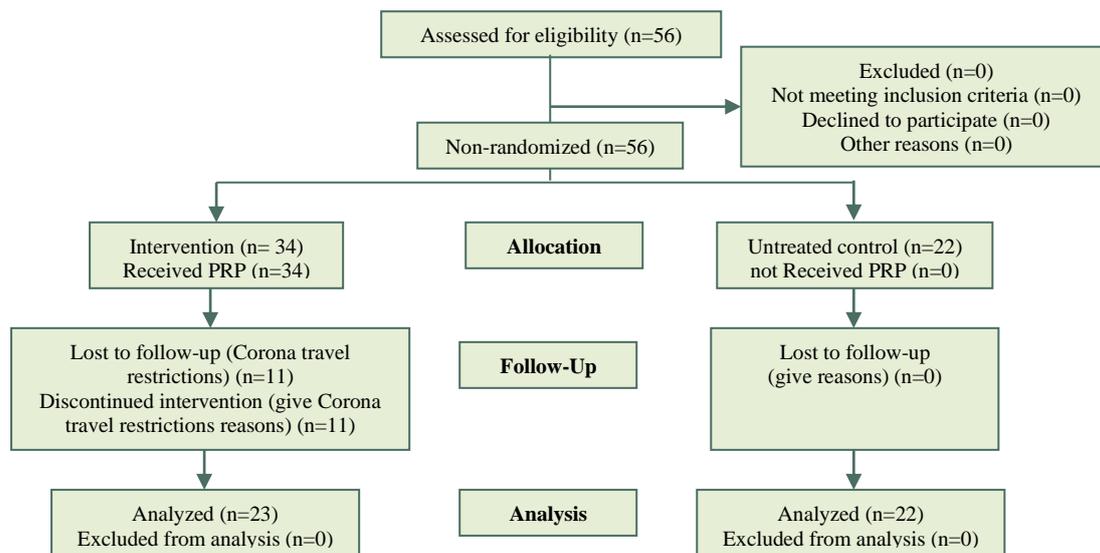

Figure 1: CONSORT flow diagram

Table 2: Post-treatment outcomes in two groups

	Intervention	Control	P-value
AMH (mean±SD)*	39.34±3.88	40.81±3.68	0.307
IVF outcome (mean±SD)*			
Oocyte	1.04±1.29	1.04±0.78	0.511
Embryo	4.21±1.31	3.68±1.42	0.197
Oocyte quality (n, %)***			0.710
M2	10 (43.5)	12 (54.5)	
M1	1 (4.5)	1 (4.5)	
GV	1 (4.5)	2 (9)	
None	11 (47.5)	7 (32)	
Embryo quality (n, %)***			0.449
A	1 (4.5)	1 (4)	
AB	3 (13)	5 (23)	
B	2 (9)	5 (23)	
All of them	1 (4.5)	0 (0)	
None	16 (70)	9 (41)	
IVF cycle outcome (n, %)***			0.003
Oocytes frozen	4 (12)	1 (4.5)	
Embryos frozen	7 (21)	10 (54.5)	
No response	6 (18)	1 (4.5)	
No embryo	6 (18)	10 (45.5)	
Chemical pregnancy (n, %)**	1 (3)	0 (0)	0.607

AMH: Anti-Müllerian hormone; IVF: In vitro fertilization
*Mann-Whitney test; **Fisher's exact test; ***Chi-square test
No Response; Dominant follicles are not obtained during ovulation stimulation treatment.
None; Failure to form of oocyte or embryo

Baseline characteristics and baseline ovarian findings (FSH, luteinizing hormone [LH], AFC [Antral follicle count], oocyte quality) were compared between the two groups. Table 1 shows no significant differences in any parameter between the two groups. However, pre-treatment AMH levels in the control group (0.40±0.76 ng/ml) were lower than the intervention group (0.62±0.49 ng/ml) (p=0.003).

Women treated with PRP had significant improvements in AMH levels compared with the control group (Table 2). Notably, the changes in AMH levels were higher following PRP (p<.001) compared with the control group (p=.001) The AMH changes indicate an increase in the intervention group compared to a decrease in the control group after two months (0.05 ± 0.34 vs -0.30 ± 0.48 respectively, p=.001) (Table 3).

The numbers of oocytes (p=0.51), the quality of oocytes (p=0.71), the quality of embryos (p=0.44) and embryos number (p=0.197) obtained from IVF did not differ between the intervention and control groups after treatment. Also the chemical pregnancy rate did not differ between the intervention and control groups (Table 2).

After treatment, there were no embryos observed in 6 (18%) individuals in the intervention group and, there were no embryos for 10 (45%) individuals in the control group, which was statistically significant.

There was one chemical pregnancy reported in the intervention group and none in the control group. There were no serious adverse effects observed in either group and there were no cases of infection.

Table 3: Pre- and post-treatment changes in two groups

Variable*	Intervention	Control	P-value
Change in AMH (ng/ml)	0.05±0.34	-0.30±0.48	0.001
Number of oocyte changes	-0.21±1.16	-0.36±0.78	0.570
Number of embryo changes	3.52±1.78	2.77±2.34	0.199

AMH: Anti-Müllerian hormone
*Data presented as mean±SD (Mann-Whitney test).

Discussion

This non-randomized controlled study compared the effect of PRP versus no intervention upon ovarian reserve parameters in women with POR. Our findings showed that one course of treatment with PRP improved AMH levels compared with no intervention. However, PRP did not have any effect on oocyte number, oocyte and embryo quality, and chemical pregnancy rate.

Interestingly, despite the fact that the basic AMH level of the control group was higher than our interventional group (p=0.003), after the intervention, the changes in the AMH level were significantly increased in the intervention group, whereas, we did not detect such difference in the control group. It could be explained that the intervention had a significant effect on ovarian reserve that was not seen in the control group. Our findings supported the results of several studies (23-30), that reported an increase in AMH levels after intra-ovarian PRP infusion in women with POR.

Pantos et al (26), Sills et al (27), and Sfakianoudis colleague (28) also reported increased serum AMH levels after intra-ovarian infusions of PRP. However, this increase in serum AMH levels was only statistically significant in the study by Pantos et al (26). Cakiroglu et al (25) reported that PRP treatment increases the AMH level.

The results of a meta-analysis of four studies, one non-randomized clinical trial and three quasi-experimental (uncontrolled before and after) studies, showed the benefits of PRP intervention on ovarian storage parameters and increased serum AMH levels (29, 30).

Aflatoonian et al (31) examined the effect of intraovarian injection of PRP on ovarian reserve factors and pregnancy outcome in women diagnosed with POI and PORs. Their study did not show a significant difference in the rate of AMH levels in the two study groups According to the results of the study by Keikha et al (21), it seems that two times 4cc PRP injection in single ovary has no effect on the outcomes (number of embryos, number of eggs, FSH, and AMH levels)in women with POR. Different ovarian PRP methods, ovulation stimulation protocols, number of PRP treatment courses, PRP injection time, follow-up time after treatment, the difference in the number of ingredients used in PRP, and small sample size, including patients and patients aged over 40 years are possible causes of differences in the results (31, 21).

Although PRP contains growth factors that are critical to cell differentiation, proliferation, angiogenesis activation, and tissue regeneration (31-34), the use of PRP in ovarian rejuvenation has not been studied in detail. Therefore, in order to evaluate the effectiveness, short-term and long-term side effects of this new treatment method, randomized clinical trial studies with a larger sample size should be considered before clinical application.

Sfakianoudis et al. (28) and Melo et al. (24) reported increases in the number of retrieved oocytes and number of embryos, and improved embryo quality after intra-ovarian PRP infusions compared to the control groups that did not receive PRP. The recent studies have shown that injecting PRP directly into the ovary can increase folliculogenesis and oocyte retrieval (35, 36).

In a study by Cremonesi et al. (37)., 5ml PRP was injected into one ovary of eight cows. In a survey by Farimany et al. (36), 12 women suffering from POR underwent ovarian stimulation, and injection of 2ml PRP, The results showed an increased AMH level. Melo et al. (24). conducted a non-randomized intervention study (PRP vs no injection). Their results showed increased AMH after treatment versus nonintervention, increased the number of collected eggs, and a higher degree of resulting embryos.

In the current study, there were no differences in the total number of retrieved oocytes, number of embryos, embryo quality, and chemical pregnancy between the PRP and control groups. . The natural temporal and biological factors affecting the process of ovulation stimulation may be the reason for the difference between the results of the present study and the mentioned studies (23, 35, 37, 38, 39).

In relation to low pregnancy rates; it can be said that due to the low number of oocytes in each puncture; the treatment plan in most patients was embryo banking and embryo transfer in future cycles was postponed after reaching the appropriate number of embryos.

Navali et al (40) showed that a single intra-ovarian of PRP injection is associated with an elevation in the number of oocytes and embryos although further evidence is required to assess the influence of PRP on the live birth rate.. Parvanov et al (41) revealed that using ovarian PRP in poor responders may be associated with a significant improvement in oocyte and embryo quality. However, the results of Tulek et al (42) study showed that intra-ovarian injection of PRP do not increase live birth rates or clinical

pregnancy rates in poor responder women. Davari Tanha et al (43) showed single-dose autologous intra-ovarian PRP injections of POR women Increase AMH, number and quality of oocytes, although these changes were not significant and did not improve the pregnancy outcomes.

We, and the previous study, conclude that there is a clear need for future controlled studies to identify patients who benefit most from PRP administration (28, 23, 40, 41, 42, 43).

The outcome of IVF following treatment and results of the above mentioned studies differ from the present study results. We believe these differences could be attributed to different ovarian PRP methods, ovulation stimulation protocols, the number of PRP treatment courses, the time of PRP injection, follow-up time after treatment, differences in the amount of material used in PRP, small sample size, the included patients and patient age above 40 years.

Limitations: The main limitation of this study is not to be a randomized clinical trial and the patients willingly chose to receive PRP or enter the untreated control group. Also there was not a placebo control group. Another limitation was the small sample size, which was insufficient to identify any potential effects associated with IVF outcomes.

To the best of our knowledge, no study has assessed the effects of PRP on blood estradiol levels in terms of the short or long term effects of PRP and it is suggested to conduct cohort studies in which estradiol levels are also evaluated.

Conclusion

In general, considering the changes in AMH levels, it could be concluded that administration of PRP resulted in more positive results for AMH outcomes.

In women with POR, autologous intrauterine PRP injections may be an alternative treatment option. Future studies with larger sample sizes and randomized prospective studies will help determine whether this intervention leads to improved clinical outcomes. Until then, autologous PRP therapy should not be recommended as part of routine treatment in women with POR.

Conflict of Interests

Authors declare no conflict of interests.

Acknowledgments

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