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The Efficacy of Ovarian Platelet-Rich Plasma Therapy on Hormonal Profiles and Pregnancy Outcomes in Women with Premature Ovarian Insufficiency

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ABSTRACT

Background & Objective: Research on the efficacy of ovarian Platelet-Rich Plasma (PRP) in enhancing fertility among women with Premature Ovarian Insufficiency (POI) is limited. This study was conducted with aim to evaluate the impact of ovarian PRP on serum levels of Follicle-Stimulating Hormone (FSH) and Anti-Müllerian Hormone (AMH), Antral Follicle Count (AFC), and pregnancy outcomes.

Materials & Methods: This interventional study was conducted at the infertility clinic of Talaqani Hospital in Tehran, Iran, in 2023. The study population involved 52 infertile women diagnosed with reduced ovarian reserve. Participants underwent PRP therapy using a 20 mg blood sample. Baseline measurements of AMH, FSH, and AFC were taken on the day of injection and reassessed post-treatment. The patients were monitored for six weeks regarding spontaneous pregnancy, and if not achieved, underwent another IVF cycle. The Wilcoxon test was used to compare median hormone levels and AFC before and after the intervention. $P<0.05$ was considered statistically significant.

Results: Significant improvements were noted post-treatment, with median FSH levels decreasing from 12.2 (IQR: 6.4) to 9.4 (IQR: 5.2) (P=0.002), and median AMH levels increasing from 0.3 (IQR: 0.5) to 0.5 (IQR: 0.8) (P=0.011). AFC also showed significant change, from a median of 2.0 (IQR: 2.0) pre-treatment to 4.0 (IQR: 4.0) post-treatment (P=0.004). Clinical pregnancy was achieved in 12 patients (23.0%), with five experiencing miscarriages or early pregnancy losses (41.6%).

Conclusion: This study demonstrates that ovarian PRP can effectively enhance ovarian function and fertility outcomes in women with POI, evidenced by improved pregnancy rates, increased levels of AMH and AFC, and decreased levels of FSH.

Keywords: Ovarian platelet-rich plasma, In-vitro fertilization, Pregnancy outcome

1. Introduction

Premature Ovarian Insufficiency (POI) is a condition characterized by a significant decrease in ovarian reserves, potentially reducing the likelihood of a successful pregnancy in women of reproductive age **(1)**. The European Society of Human Reproduction and Embryology has identified three main criteria for diagnosing POI: primary or secondary amenorrhea lasting more than four months before the age of 40, an Follicle-Stimulating Hormone (FSH) level above 25 mIU/ml, and low serum levels of Anti-Müllerian Hormone (AMH), which reflect the size of the primordial follicle pool **(2)**.

Several mechanisms have been identified as primary causes of POI, including early follicular depletion, destruction of oocyte pools, and ovarian syndrome, which is characterized by high FSH levels and low Antral Follicle Count (AFC) **(3,4)**. These factors play a significant role in the onset of POI **(5,6)**. Pregnancy is typically rare among women with POI, and effective treatment is crucial **(2)**. While oocyte donation is often considered the first-line treatment, it faces legal and ethical constraints that limit its accessibility. Other treatments, such as Hormone Replacement Therapy (HRT) and In Vitro Fertilization (IVF), have been explored, but often with limited success **(2)**. Ovarian Platelet-Rich Plasma (PRP), a recently developed

intervention, aims to improve pregnancy rates in women with POI. Previous studies suggest that ovarian PRP can offer several benefits, including lowering FSH levels and increasing AMH and AFC **(7,8)**. Evidence also indicates that this treatment could potentially boost pregnancy rates in women with POI by up to 20% **(7,9)**.

However, there is limited research on the efficacy of ovarian PRP and its effects on the pregnancy outcomes for women with POI. Therefore, the current study was conducted with aim to investigate the impact of ovarian PRP on the serum levels of FSH and AMH, as well as the number of AFC before and after treatment. Additionally, pregnancy outcomes were assessed among a group of women with POI who were referred to the infertility clinic at Talaqani Hospital in Tehran, Iran, in 2023.

2. Materials and Methods

This interventional study was conducted on 52 infertile women diagnosed with reduced ovarian reserve who underwent ovarian PRP therapy. The participants were randomly selected from individuals referred to the infertility clinic at Talaqani Hospital in Tehran, Iran, over a six-month period in 2023.

The inclusion criteria were participants aged over 40 years who had AMH levels ranging from 0.5 to 1.1 ng/ml, an AFC of less than 5, and FSH levels exceeding 10 mIU/ml. Additionally, candidates were required to have a minimum of one year of infertility history, at least one ovary, and at least one unsuccessful cycle of Assisted Reproductive Technology (ART). Exclusion criteria included patients with endometriosis or a history of uterine or ovarian abnormalities.

For the preparation of PRP, 20 mg of blood was drawn from each patient under sterile conditions and centrifuged for 8 minutes at 830 g. Using a 16G needle attached to a 5 ml syringe, the PRP was extracted directly from the tubes without removing the growth factor-rich blood clot. Approximately 2-4 cc of PRP was collected from each of the first and second tubes, yielding a total of 4-8 cc. This PRP solution was then transferred to a separate tube and gently agitated for 30-60 seconds. The intraovarian PRP injection was conducted in the operating room, within two hours of PRP preparation. The procedure involved transvaginal injection of PRP into at least one ovary using a 17G needle, 35 cm in length, under ultrasound guidance. The injections were performed multiple times, with sessions spaced every other month, totaling 2-3 treatments. Despite potential challenges due to the smaller size and fibrotic nature of ovaries in patients with advanced maternal age and poor ovarian reserve, injections were administered by creating new levels within the ovary for distribution. Patients were then transferred to the recovery room, and after 30-40 minutes, discharged on the same day. PRP injections were scheduled within 10 days post-menstruation. On the day of the injection, baseline measurements of AMH, FSH, and AFC were taken. These parameters were measured again following the PRP treatment. Patients were monitored regarding spontaneous pregnancy for six weeks. If no pregnancy occurred, the patient underwent another IVF cycle to reassess fertility post-PRP treatment.

The primary outcome was clinical pregnancy, which was determined by the presence of a gestational sac or fetal heartbeat detectable through ultrasound, or by elevated hCG levels in the blood. The secondary outcome was the miscarriage rate, defined as the spontaneous loss of a clinical pregnancy before 20 weeks of gestation. Additionally, the effects of PRP therapy on median levels of FSH, AFC, and AMH were assessed before and after the intervention.

Data were evaluated using descriptive statistics. Parametric variables conforming to a normal distribution were summarized with mean and standard deviation, whereas non-parametric variables were reported using median and interquartile range. Dichotomous variables were expressed as counts and percentage frequencies. To determine the effectiveness of PRP, we analyzed changes in the median values of FSH, AMH, and AFC before and after PRP. Due to the non-normal distribution of these variables, the non-parametric Wilcoxon test U test was utilized for analysis. Statistical analyses were conducted using Stata software (Version 17.0, StataCorp, College Station, Texas, USA), and $P<0.05$ was considered statistically significant.

3. Results

The mean age of the study participants was 39.1±3.5 years. The mean Body Mass Index (BMI) was 29.8±5.0 kg/m^2. The median seminal concentration was 50.0, and the median proportion of sperm with progressive motility was 27.4%, with an Interquartile Range (IQR) of 29.5. Additionally, normal sperm morphology was observed in only two patients (15.4%) (Table 1).

Table 2 compares the levels of FSH, AMH, and AFC before and after PRP therapy. The median FSH level for all participants was 12.2 (IQR: 6.4) before ovarian PRP treatment and decreased to 9.4 (IQR: 5.2) after treatment, with this difference statistically significant (P=0.002). The median AMH level was 0.3 (IQR: 0.5) before the intervention and increased to 0.5 (IQR: 0.8) after PRP therapy, also showing a statistically significant improvement (P=0.011). A significant change was observed in AFC, with a median of 2.0 (IQR: 2.0) before ovarian PRP and 2.0 (IQR: 4.0) after ovarian PRP (P=0.004) (Table 2).

For the 34 patients included in the study, only one cycle of treatment was performed for 66.4%, while 12 patients underwent two cycles (23.5%), and 5 patients (9.8%) had three or more cycles . Among these cycles, embryo formation was observed in 16 patients (30.8%). administered PRP treatment was

administered to 44 patients, while eight patients received PRP twice. Clinical pregnancy was achieved in 12 patients (23.0%), with five experiencing miscarriages and early pregnancy losses (41.6%) (Table 3).

Table 1. Study Participants' Baseline Characteristics

Characteristics	N=52
Age, Mean (SD), year	39.1(3.5)
BMI, Mean (SD), kg/m²	29.8(5.0)
Seminal fluid analysis	
Concentration, Median (IQR)	50.0(35.5)
Morphology shape, n (%)*	
Abnormal	44(84.6%)
Normal	8(15.4%)
Progressive motility, Median (IQR)	27.4(29.5)

BMI: Body Mass Index, SD: Standard Deviation, * Morphology of sperms was missing in 5 patients.

Table 2. Comparison of FSH, AMH, and AFC Levels in Infertile Women Before and After Ovarian PRP

Characteristics	Before	After	P-value
FSH, Median (IQR), IU/L	12.0 (6.4)	9.4 (5.2)	0.002
AMH, Median (IQR), ng/mL	0.3 (0.5)	0.5 (0.8)	0.011
AFC, Median (IQR)	2.0 (2.0)	2.0 (4.0)	0.004

FSH: Follicle-Stimulating Hormone, AMH: Anti-Müllerian Hormone, AFC: Antral Follicle Count, IQR= Interquartile range

Table 3. Ovarian PRP Treatment Characteristics and Reproductive Outcomes in Patients

Outcome	N=52
N of cycle	
1 cycle	34(66.7%)
2 cycles	12(23.5%)
3 or more	5(9.8%)
Cycle outcome	
Failure	36(69.2%)
Embryo formation	16(30.8%)
N of ovarian PRP	
One time	44(84.6%)
Two times	8(15.4%)
Clinical pregnancy, n (%)	12(23.0%)
Miscarriage rate, n (%)	5(41.6%)

4. Discussion

POI is linked to a reduced likelihood of achieving pregnancy and having a successful live birth **(9)**. Typically, the first treatment option for women with POI is oocyte donation **(2)**. However, this approach faces considerable challenges, including societal hesitations about having a child with shared genetic material, as well as legal restrictions that make oocyte donation illegal in many parts of the world **(2)**. Consequently, alternative treatments such as hormone therapy, exogenous ovarian stimulation, and ovarian PRP have been explored to enhance pregnancy outcomes for women with POI **(10-12)**. The purpose of the present study was to evaluate the effects of ovarian PRP on pregnancy outcomes in women with POI. Additionally, we monitored changes in the serum levels of FSH, AMH, and AFC before and after PRP treatment. Our findings revealed 12 clinical pregnancies, although five resulted in early miscarriage. Notably, there was a significant increase in the median levels of AMH and AFC post-PRP compared to their pre-PRP values. Furthermore, PRP treatment was associated with a significant reduction in FSH levels.

The pregnancy rate observed in this study was 23.0%, which is consistent with previous research findings. Tremellen et al. reported a clinical pregnancy rate of 20% in their study population, demonstrating a similar level of effectiveness of ovarian administration of Platelet-Rich Plasma (PRP) in women with severe diminished ovarian reserve **(13)**. Moreover, a slightly lower pregnancy rate of 17.5% reported by Cakiroglu et al. indicates modest variability but generally consistent results across studies **(7)**. In another related study, Aflatoonian et al. investigated clinical pregnancy outcomes in women with POI who

underwent ovarian PRP treatment and observed a pregnancy rate of 20.5%, with 12.9% resulting in live births **(14)**. This underscores the potential efficacy of PRP treatment in improving fertility outcomes for women with POI. Additionally, Melo et al., conducted a study on the use of autologous Platelet-Rich Plasma (PRP) in women with low ovarian reserve undergoing fertility treatment and showed a higher clinical pregnancy rate in women with low ovarian reserve who received PRP compared to the control group **(15)**. These findings are supported by results from a non-randomized study that compared the pregnancy rates between women who received PRP in their IVF treatment and those who did not. PRP contains numerous active substances such as growth factors Transforming Growth Factor Beta (TGF-β), Insulin-Like Growth Factor (IGF), Platelet-Derived Growth Factor (PDGF), Epidermal Growth Factor (EGF), Basic Fibroblast Growth Factor (bFGF), Vascular Endothelial Growth Factor (VEGF) and cytokines like Interleukin 1 Beta (IL-1β) and IL-8 **(16)**. The presence of these substances can explain the follicular activation observed following intraovarian PRP injections. This treatment can activate the abundant primordial follicles commonly found in the ovaries, accelerating their development into primary follicles, secondary follicles, and eventually antral follicles, which can produce a mature oocyte **(17,18)**. Another proposed mechanism for PRP's effectiveness involves reversing ovarian hypoperfusion and enhancing oxygen delivery. This improvement in oxygen supply, along with the clearance of Reactive Oxygen Species (ROS), is facilitated by mediators released by PRP **(19)**. These changes could significantly contribute to the recovery and health of ovarian follicles **(20)**.

The results of our study also observed significant changes in AMH and FSH levels. After PRP treatment, the serum level of AMH increased significantly, while the level of FSH decreased. Our findings are consistent with previous studies, which have similarly shown significant changes in AMH and FSH levels favoring patients treated with ovarian PRP **(7,21)**. The observed significant changes in the serum levels of AMH and FSH in our study present a potential pathway to explain the increased pregnancy rates in women with POI who were treated with ovarian PRP. Notably, an increase in AMH and a decrease in FSH could indicate a rejuvenation of ovarian function. The reduction in FSH levels is particularly crucial as it may contribute to the regeneration of ovarian tissue and enhance responsiveness **(20)**. This downregulation of gonadotropin receptors, because of decreased FSH levels, could potentially restore the hormonal balance necessary for ovulation and fertility **(20)**.

The current study represents one of the few efforts to investigate the impact of a newly developed treatment on the pregnancy outcomes of women with POI in Iran. These findings are valuable as they add to the growing body of evidence supporting the efficacy of ovarian PRP for treating POI, thereby enhancing our understanding of its potential benefits. However, the interpretation of these results must be approached with caution due to several limitations. The main constraints include a small sample size and a limited follow-up period. Additionally, the absence of a control group precludes direct comparisons between women who received ovarian PRP and those who did not, potentially affecting the robustness of the conclusions drawn from this study.

5. Conclusion

As evidenced by the results of the present study, notable improvement was observed in pregnancy rates, alongside higher level of AMH and AFC, and lower levels of FSH among women with POI treated with ovarian PRP. These findings suggest that ovarian PRP treatment may effectively enhance ovarian function and fertility in women with POI. However, while these results are promising, further research with larger sample sizes and controlled study designs is essential to confirm the efficacy and safety of ovarian PRP, thereby supporting its potential as a viable treatment option for improving reproductive outcomes in this patient population.

6. Declarations

Acknowledgments

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Ethical Considerations

Prior to enrollment, the study's objectives and procedures were fully explained to the participants, and all of them provided written informed consent. The study was approved by the ethics committee and review board of Shahid Beheshti University of Medical Sciences.

Authors' Contributions

S.S contributed to project administration and conceptualization. M.H, S.H, M.M, and F.R performed writing – original draft and formal analysis. H.Z participated in review & editing. M.N contributed to project administration and supervision. All authors read and approved the final manuscript and took responsibility for the integrity of the data.

Conflict of Interest

The authors declare no conflict of interest.

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