

Effects of intraovarian injection of autologous platelet-rich plasma on ovarian rejuvenation in women with primary ovarian insufficiency

Abstract

Background and study aim: Primary Ovarian insufficiency, which is described by significantly reduced ovarian reserve, menstrual irregularity, or amenorrhea earlier than 40 years of age, affects 1% of women at reproductive age. Recently, platelet-rich plasma (PRP) has been widely used in regenerative treatment in different fields, as it is rich with cytokines and growth factors. Accumulation of platelet in a tissue stimulates cell proliferation and tissue regeneration through protein secretion in response to cytokines, and growth factors. Recently, it has been investigated in the cases of ovarian insufficiency. This study aimed to evaluate whether the intra-ovarian injection of autologous PRP would improve ovarian reserve through ovarian rejuvenation thus causing spontaneous pregnancy or menstrual recovery in women with primary ovarian insufficiency.

Patients and Methods: This Quasi-Experimental study was conducted in the department of Obstetrics & Gynecology, Suez Canal University hospital. It was conducted on 38 Women of childbearing period presenting at the outpatient clinic of the Department of Obstetrics and Gynecology complaining of Primary ovarian insufficiency diagnosed according to ESHRE guideline.

Results: Our study showed that FSH levels rose considerably, indicating a reduction in ovarian reserve. Although AMH levels remained relatively stable, there was a slight decrease in the median value, which could suggest a potential trend towards diminished reserve. AFC demonstrated a statistically significant decrease, with an increasing number of patients exhibiting 0 or 1 follicle after a two-month period. These results indicate a **significant deterioration in ovarian reserve markers over 2 months**, especially reflected by a rise in FSH and a drop in AFC.

Conclusion: In contrast to previous reports demonstrating improved ovarian reserve markers following PRP treatment, this study did not observe a favorable change in ovarian reserve two months post-intraovarian PRP in women with established POI. The findings underscore the need for longer follow-up, larger sample sizes, and standardization of PRP protocols. Future research should focus on identifying patient subgroups most likely to benefit from PRP and establishing biological markers predictive of response.

Keywords: platelet-rich plasma, ovarian insufficiency, Antral follicular count, antimullerian hormone

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Mahmoud Ahmed Mohamed Greash,¹
Mahmoud Alalfy,² Amr Abbassy,² Alaa Hamed
Alarshal,³ Asmaa MM Abdellah,¹ Seif Ali,⁴
Ahmed Khalil,⁵ Waleed F Gharib¹

¹Obstetrics and Gynecology department, Suez Canal University, Egypt

²Reproductive health and family planning, National Research Centre, Egypt

³Obstetrics and Gynecology department, Alazhar University, Egypt

⁴Obstetrics and Gynecology department, Assiut University, Egypt

⁵Obstetrics and Gynecology department, university hospital Schleswig Holstein, Campus Lubeck, Germany

Correspondence: Mahmoud Alalfy, Obstetrics and Gynecology Department, National Research Centre, Egypt, Tel 01002611058

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Abbreviations: FSH, follicle stimulating hormone; AMH, antimullerian hormone; PRP, platelet rich plasma; AFC, antral follicular count; OPU, oocyte pick up

Introduction

Women with primary ovarian insufficiency (POI) are considered the main challenges of reproductive science. POI, which is described by significantly reduced ovarian reserve, menstrual irregularity, or amenorrhea earlier than 40 years of age, affects 1% of women at reproductive age.¹

At present, egg donation is the only treatment option for women with POI, and infertile couples who desire to have their own genetic offspring do not usually welcome that. Recently, platelet-rich plasma (PRP) has been widely used in regenerative treatment in different fields such as orthopedics, dermatology, dentistry, and aesthetic surgery.^{2,3}

PRP is platelet-rich blood plasma supplemented with cytokines and growth factors. Accumulation of platelet in a tissue stimulates

cell proliferation and tissue regeneration through protein secretion in response to cytokines, and growth factors lead to revert the cellular damage and tissue rejuvenation.⁴

In the field of infertility, PRP was primarily used for the treatment of thin endometrium and recurrent implantation failure.^{5,6}

Recently, it has been investigated in the cases of ovarian insufficiency.^{7,8} Since the studies which assessed the PRP beneficial effects on ovarian parameters are limited, So the aim of this study to evaluate whether the intra-ovarian injection of autologous PRP would improve ovarian reserve through ovarian rejuvenation thus causing spontaneous pregnancy or menstrual recovery in women with primary ovarian insufficiency.

Patients and methods

Type of study: A Quasi-Experimental study.

Site of study: The department of Obstetrics & Gynecology, Suez Canal University hospital.

Study population: Women of childbearing period presenting at the outpatient clinic of the Department of Obstetrics and Gynecology complaining of Primary ovarian insufficiency diagnosed according to ESHRE guideline.

Criteria of selection

Inclusion criteria: POI patients were diagnosed according to ESHRE guideline: onset prior to 40 years of age, oligo-/amenorrhea for at least 4 months, and an elevated FSH level > 25 IU/l on two occasions > 4 weeks apart.

We excluded the subjects who had:

- a) Body mass index (BMI) above 30 or less than 18.
- b) Autoimmune diseases, thrombophilic disorders.
- c) Ovarian insufficiency secondary to sex chromosome etiology, sexually transmitted diseases, tubal factor infertility, endocrine disorders such as thyroid dysfunction, endometriosis.
- d) Previous major lower abdominal surgery and pelvic adhesions.

Study methods

Data was collected in pre organized data sheet by the researcher from patients fulfilling inclusion and exclusion criteria. All patients of our study were subjected to the following:

- 1) **Thorough history taking**, including age, parity, obstetric history, detailed menstrual history, previous surgery, chronic illness, past history of any operation
- 2) **Laboratory investigations:** including CBC, Hormonal profile (FSH , LH , E2 , AMH , TSH , Prolactin) was performed three times: prior to PRP injection and two consecutive months after the first PRP injection
- 3) **TV US examination:** Antral follicular count was also measured

Sample preparation

PRP was prepared from autologous blood using the manufacturer's instruction (Rooyagen, Tehran, Iran). Briefly, for each PRP infusion, approximately 20 ml of peripheral venous blood was drawn in the syringe that contained 3 ml of acid citrate A anticoagulant solution (ACD-A) (Arya Mabna Tashkhis, Iran) and centrifuged immediately at 1600g for 10 min.

The blood was divided into three layers including red blood cells at the bottom, a buffy coat layer, and cellular plasma as the supernatant. The plasma layer and buffy coat were transferred to another tube and centrifuged at 3500g for 5 min to achieve 3 ml PRP of 3 to 5 times higher than basal blood samples.

Prepared PRP was stored for 1 h at a temperature of 4°C before injection, and PRP activation was performed using calcium gluconate (CG) in a 1:9 ratio.

Blood products such as PRP fall under the prevue of FDA's Center for Biologics Evaluation and Research (CBER). CBER is responsible for regulating human cells, tissues, and cellular and tissue-based products. The regulatory process for these products is described in the FDA's 21 CFR 1271 of the Code of Regulations. Under these regulations, certain products including blood products such as PRP are exempt and therefore do not follow the FDA's traditional regulatory pathway that includes animal studies and clinical trials.

Intraovarian injection

The injection was randomly scheduled in POI women with amenorrhea, whereas in POI women who were oligomenorrheic, PRP injection was done 10 days after the beginning of menstrual bleeding. PRP injection was done according to the previously defined method.^{9,10}

The injection of PRP is mostly an empirical process, Injecting PRP into the ovaries is a technique based on the method of trans-vaginal paracentesis employed during the oocyte pick up (OPU) procedure, Minimal sedation was administered to the patients. Injection and diffusion into the ovarian stroma were performed under a non-surgical, trans-vaginal ultrasound-guided multifocal intramedullary procedure, allowing approximately 4 ml PRP for each of both ovaries.

In a more detailed description, the ovaries were identified with the guidance of trans-vaginal ultrasonography. Following identification, a needle was inserted, penetrating the ovarian tissue, accompanied by a resistance felt by the practitioner. Prior to initiating the injection procedure, the needle guide was aligned with the ovaries to ensure that any surrounding structures, such as vessels, were not compromised.

Taking into account the anatomical position of the ovary, a 17-gauge single lumen needle was employed, moving across the central part of the ovary, carefully covering the distance from one side to the other, practicing extreme caution so as to avoid penetrating the germinal epithelium of the outer wall of the ovary into the peritoneal cavity.

In the case of POI, a quantity of the injected PRP leaked into the peritoneal cavity due to atrophic ovaries with decreased volume.

By the end of the procedure, accurate ultrasonography was performed to evaluate the pelvic area concerning the amount of leakage and vascular integrity.

The majority of women received the second PRP injection with a twofold increase in the dosage to 3ml, 3 months after the first injection (Figure 1).

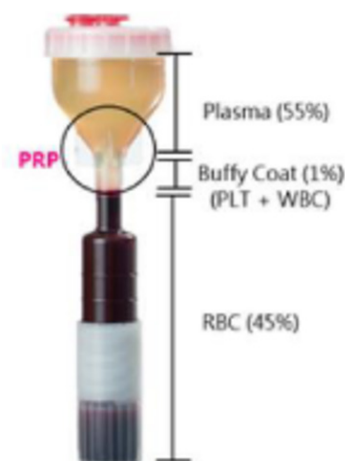


Figure 1 PRP preparation.

PRP insertion:

- 1) Timing of insertion: day 9 of the cycle and repeated after 48 hours if no response
- 2) Needle used: walles needle
- 3) Amount of PRP 0.5-1 ml

Statistical analysis

Data were coded and entered into the computer statistical program. All statistical analyses were performed using the Statistical Package for Social Science (SPSS) version (25).

- Descriptive statistics was applied in numerical form (mean, SD or percentages) to describe the quantitative variables.
- Diagrammatic and tabular forms were used -when appropriate- to describe the qualitative variables.
- Data presentation was performed via tables and graphs. Qualitative data was presented as numbers and percentages while quantitative data as mean $\pm$ Standard Deviation.
- Kolmogorov test was used to test normality.
- Parametric and non-parametric tests were used as required.
- (T) test was used for normally distributed quantitative variables while Mann-Whitney test was used for quantitative variables that are not normally distributed.
- Chi square and Fisher's exact tests were used for qualitative variables.
- P value of <0.05 was considered statistically significant.

Results

In this study, we assessed and compared the effect of intraovarian PRP injection on ovarian rejuvenation in women with Primary Ovarian Insufficiency (POI).

We included 38 subjects (3 did not complete the study so the final number was 35) diagnosed according to ESHRE guideline: onset prior to 40 years of age, oligo-/amenorrhea for at least 4 months, and an elevated FSH level > 25 IU/l on two occasions > 4 weeks apart.

Table 1 shows that the studied group comprises women in their early 30s, with a relatively long mean duration of infertility (6 years).

Table 1 Distribution of the studied cases according to different parameters (n = 35)

	No. (%)
Age	
Min. – Max.	25.0 – 39.0
Mean $\pm$ SD.	32.03 $\pm$ 3.89
Median (IQR)	32.0 (29.0 – 35.0)
Duration of infertility	
Min. – Max.	2.0 – 17.0
Mean $\pm$ SD.	6.09 $\pm$ 3.48
Median (IQR)	5.0 (3.0 – 8.50)
Previous pregnancy	
0	21 (60.0%)
1	11 (31.4%)
2	3 (8.6%)
Pregnancy outcome	
No	35 (100.0%)
Yes	0 (0.0%)

It also shows that a majority had never been pregnant, and none achieved pregnancy, suggesting a population with significant reproductive challenges.

Table 2 shows that FSH levels rose considerably, indicating a reduction in ovarian reserve.

Table 2 Comparison between baseline and after according to FSH,AMH and AFC (n = 35)

	Baseline (n = 35)	After 2 Month (n = 35)	Test of Sig	P
FSH				
Min. – Max.	25.0 – 60.0	23.0 – 58.0	Z=	0.035*
Mean $\pm$ SD.	32.03 $\pm$ 9.10	32.91 $\pm$ 9.64	2.111*	
Median (IQR)	28.0 (26.0 – 36.5)	30.0 (26.0 – 40.0)		
AMH				
Min. – Max.	0.001 – 0.40	0.001 – 0.50	Z=	0.139
Mean $\pm$ SD.	0.16 $\pm$ 0.14	0.14 $\pm$ 0.13	1.48	
Median (IQR)	0.20 (0.01 – 0.25)	0.10 (0.01 – 0.20)		
AFC				
0	5 (14.3%)	12 (34.3%)	MH=	0.001*
1	15 (42.9%)	17 (48.3%)	34.500*	
2	7 (20.0%)	3 (8.6%)		
3	8 (22.9%)	3 (8.6%)		
Mean $\pm$ SD.	1.51 $\pm$ 1.01	0.91 $\pm$ 0.89	Z=	0.001*
Median (IQR)	1.0 (1.0 – 2.0)	1.0 (0.0 – 1.0)	3.202*	

Although AMH levels remained relatively stable, there was a slight decrease in the median value, which could suggest a potential trend towards diminished reserve.

AFC demonstrated a statistically significant decrease, with an increasing number of patients exhibiting 0 or 1 follicle after a two-month period.

These results indicate a significant deterioration in ovarian reserve markers over 2 months, especially reflected by a rise in FSH and a drop in AFC.

The absence of improvement in AMH or AFC suggests that no beneficial intervention took place, or the intervention was not effective.

Discussion

This study aimed to evaluate the short-term effects of intraovarian autologous platelet-rich plasma (PRP) injections on ovarian reserve in women diagnosed with primary ovarian insufficiency (POI). The results demonstrated a significant increase in FSH, a significant reduction in AFC, and a non-significant decline in AMH two months post-PRP administration. Collectively, these findings suggest no favorable impact of PRP on ovarian reserve within this short follow-up duration and may even indicate further deterioration in some patients.

These results stand in contrast to numerous studies that have reported beneficial effects of intraovarian PRP on ovarian function in women with POI or diminished ovarian reserve (DOR). For instance,¹¹ described a case series where women with POI treated with intraovarian PRP exhibited resumed menses and even spontaneous pregnancies. Similarly,¹⁰ conducted a pilot study on women with POI and observed significant increases in AMH, decreased FSH, and resumption of follicular activity on ultrasound within 2–4 months following PRP injection.

Moreover,¹² conducted a prospective study on 311 women with low ovarian reserve, where PRP was shown to significantly improve AMH and AFC in a majority of participants within three months. Notably, clinical pregnancy was achieved in 24.1% of participants. Also demonstrated similar results, reporting significant increases in both

AMH and AFC, suggesting that PRP may enhance the recruitment and activation of dormant follicles.⁵

In contrast, our study demonstrated a statistically significant increase in FSH levels and significant reduction in AFC, which are markers typically associated with worsening ovarian function. The lack of AMH improvement and the higher percentage of women with zero follicles post-treatment may indicate a non-responder group or a potentially delayed response. This may be explained by multiple factors:

- 1) **Short follow-up period:** Most successful studies reported outcomes at three or more months post-PRP injection, suggesting that ovarian tissue regeneration may require a longer period to reflect in measurable hormone or ultrasound changes.
- 2) **Severity of POI:** Our study population consisted entirely of POI patients with very low AMH levels (mean 0.16 ng/mL) and a high percentage with no antral follicles at baseline. This population represents a more severe form of ovarian insufficiency compared to women with DOR or advanced age, who may respond more readily to PRP.
- 3) **PRP protocol variability:** The lack of standardization in PRP preparation, including platelet concentration, activation method, and injection volume, may also influence outcomes. For example,⁹ used activated PRP and reported better responses in follicular growth and even IVF success in some patients.
- 4) **Individual response variability:** Genetic and epigenetic factors likely modulate individual response to regenerative therapies such as PRP. Some studies, like that by Molina et al.,¹³ highlighted the presence of responders and non-responders among women receiving PRP, emphasizing the need for biomarkers to predict treatment efficacy.
- 5) **Absence of a control group:** While our study utilized a pre-post comparison design, the absence of a placebo control group limits the ability to attribute changes solely to PRP.

Despite these findings, it is important to recognize that PRP therapy is still in its experimental stages and current evidence is derived mainly from observational studies and small pilot trials. A systematic review by⁷ highlighted the promising but still inconclusive role of PRP in ovarian rejuvenation, calling for more well-designed randomized controlled trials (RCTs).

Conclusion

In contrast to previous reports demonstrating improved ovarian reserve markers following PRP treatment, this study did not observe a favorable change in ovarian reserve two months post-intraovarian PRP in women with established POI. The findings underscore the need for longer follow-up, larger sample sizes, and standardization of PRP protocols. Future research should focus on identifying patient subgroups most likely to benefit from PRP and establishing biological markers predictive of response.

Recommendations

- 1) **Conduct larger Randomized Controlled Trials (RCTs):** Most existing studies, including ours, are observational or pilot in nature. Future research should focus on multicenter RCTs with larger sample sizes to establish the efficacy and safety of intraovarian PRP with high-level evidence.

- 2) **Include control or placebo groups:** To differentiate the actual effects of PRP from placebo or natural ovarian fluctuations, studies must include well-matched control groups (e.g., saline injection or no treatment) and consider a double-blind design.
- 3) **Extend the follow-up period:** A short follow-up (e.g., 2 months) may not be sufficient to detect the regenerative effects of PRP. Future studies should include follow-ups at 3, 6, and 12 months to assess both short- and long-term impacts on ovarian reserve, follicular activity, and pregnancy outcomes.
- 4) **Standardize PRP preparation protocols:** The variability in platelet concentration, activation methods, and injection techniques complicates comparison across studies. It is crucial to establish standardized protocols for PRP preparation and administration.
- 5) **Define response criteria and stratify patients:** Future studies should define clear criteria for responders vs. non-responders and perform subgroup analyses. Stratification based on age, baseline AMH/AFC, duration of POI, and presence of autoimmune markers may help identify which patients are most likely to benefit.
- 6) **Investigate mechanisms of action:** More basic science and translational studies are needed to elucidate the molecular and cellular mechanisms by which PRP may affect folliculogenesis and ovarian regeneration. Studies should evaluate angiogenesis markers, inflammatory mediators, and gene expression changes post-PRP.
- 7) **Evaluate functional and clinical outcomes:** In addition to hormonal markers and AFC, future trials should assess functional outcomes such as:
 - a. Return of menses
 - b. Ovulation rates
 - c. Pregnancy rates (natural or assisted)
 - d. Live birth rates
- 8) **Assess safety and adverse events:** While PRP is considered autologous and safe, comprehensive monitoring for local and systemic adverse effects is essential, especially with repeated injections or in patients with autoimmune etiologies of POI.
- 9) **Explore combination therapies:** Some studies suggest that combining PRP with other regenerative therapies (e.g., stem cells, ovarian tissue fragmentation, hormonal priming) may enhance outcomes. These combinations warrant controlled evaluation.
- 10) **Cost-effectiveness analysis:** Considering the potential for clinical application, future studies should include economic evaluations comparing PRP therapy to current options (e.g., oocyte donation, hormone replacement) to guide clinical and policy decisions.

Ethics

Concerning this study, the following ethical considerations were taken:

- i. Agreements from the responsible authority will be obtained.
- ii. Approval from Research Ethics committee of the Suez Canal University, Faculty of Medicine was obtained before starting the fieldwork.

- iii. Informed consent was obtained from participants with explanation of study aim and procedures were done in the study in simple way.
- iv. Participants had the right to refuse to participate in the study.
- v. Participants had the right to withdraw from the study at any time without explaining reasons and without conflict with their work.
- vi. The maneuvers may carry minimal risks and it was dealt with.
- vii. Participants' data was kept confidential, and any data manipulation or transfer was done using codes.
- viii. Dissemination of results to stakeholders

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Conflicts of interest

The authors have no conflicts of interest.

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