

## Ovarian rejuvenation by PRP (Platelet-Rich Plasma)

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### Abstract:

**Background:** Ovarian rejuvenation is a potential therapeutic approach aimed at enhancing ovarian function and improving fertility results in women with poor ovarian reserve (POR). **Aim:** To compare PRP and normal saline injections impacts on (FSH-LH-AMH-E2) & AFC in POR patients. **Methods:** This pilot multi-center study included 20 patients with poor ovarian response (POR). Patients underwent laparoscopy, with Group A receiving an autologous PRP injection and Group B receiving a normal saline injection. Hormone levels, including FSH, LH, estradiol, and AMH, were monitored before and every month after PRP injection for a duration of 6 months. Antral follicles count was also assessed prior and following PRP treatment. **Results:** According to the ovulation status of Group A after PRP injection, out of the 10 participants, 3 (30%) ovulated, while 7 (70%) did not ovulate. Antral follicular count post treatment showed a significant negative correlation with FSH ( $p < 0.001$ ) and LH ( $p = 0.002$ ). Antral follicular count post treatment showed a significant positive correlation with E2 ( $p = 0.001$ ) while no significant correlation with age and AMH. **Conclusions:** PRP therapy leads to a substantial decrease in LH and FSH concentrations. Additionally, PRP treatment leads to a substantial elevation in estradiol (E2) levels. Antral follicle count (AFC), a marker of ovarian reserve and follicular growth, significantly increases after PRP therapy, suggesting enhanced follicular development.

**Keywords:** Ovarian; Rejuvenation; Platelet –Rich Plasma.

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## Introduction

Ovarian rejuvenation is a potential therapeutic approach aimed at enhancing ovarian function and improving fertility results in POR women. POR is characterized by reduced ovarian follicle quantity and quality, leading to difficulties in achieving successful pregnancies. Infertility is often a problem for women with advanced maternal age, a history of poor ovarian response, or abnormal ovarian reserve test findings. Therefore, exploring novel interventions such as platelet-rich plasma (PRP) holds promise for improving ovarian function and restoring fertility<sup>(1)</sup>.

PRP, a concentrated form of platelets derived from the patient's own blood, has gained attention as a potential regenerative therapy in various medical fields. PRP contains numerous growth factors, cytokines, and bioactive proteins that can stimulate tissue repair and regeneration. In the context of ovarian rejuvenation, PRP is hypothesized to promote follicular growth, enhance ovarian blood supply, and improve overall ovarian health<sup>(2)</sup>. The rationale behind PRP therapy lies in its ability to stimulate the body's own healing mechanisms to rejuvenate the ovaries<sup>(3)</sup>.

Previous studies have demonstrated the regenerative potential of PRP in various tissues, such as musculoskeletal, skin, and hair. The application of PRP has shown promising results in promoting tissue regeneration, neovascularization, and collagen synthesis. Based on these findings, researchers and clinicians have begun investigating the potential benefits of PRP in improving ovarian function and fertility outcomes<sup>(4)</sup>.

PRP usage in ovarian rejuvenation is an emerging field of research, and its underlying mechanisms of action are still being elucidated. It is believed that PRP acts through multiple pathways, including promoting angiogenesis, modulating inflammation, and enhancing follicular development. By delivering a concentrated

dose of growth factors and bioactive molecules directly to the ovaries, PRP may create an optimal microenvironment for follicular growth and maturation<sup>(5)</sup>.

The safety and feasibility of PRP therapy in the field of reproductive medicine have been explored in recent years. Studies have reported minimal adverse events associated with PRP injections, making it a potentially safe intervention for ovarian rejuvenation. However, further research is needed to establish the optimal PRP preparation protocols, injection techniques, and treatment regimens to maximize its efficacy and safety in POR patients<sup>(6)</sup>.

Understanding the effects of PRP on ovarian rejuvenation is crucial in assessing its potential as a therapeutic option for women with POR. Hormonal balance plays a pivotal role in follicular development, ovulation, and overall reproductive function. By monitoring changes in follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol, and anti-Mullerian hormone (AMH), we can gain insights into how PRP may modulate this serum hormone level of (FSH-LH-AMH-E2) and potentially improve ovarian function.

Therefore, this study aimed to compare the effects of PRP and normal saline injections on ovarian rejuvenation in POR patients.

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## Patients and methods:

This pilot multi-centre study compared Platelet-rich plasma (PRP) with normal saline for ovarian rejuvenation. Women with POR who matched at least two of the three Bologna criteria released by the European Society for Human Reproduction and Embryology (ESHRE) in 2011 were included in the research. Participants were selected from Benha University Hospital and other Gynecological centers between May 2022 and May 2023. The study was approved by the Local Ethics Committee on Research

involving Human subjects of Benha Faculty of Medicine.

**Inclusion Criteria** were patients with poor ovarian response (POR) who met at least two of the following three Bologna criteria: Advanced maternal age ( $\geq 40$  years). Previous poor ovarian response (canceled cycles or  $\leq 3$  oocytes with a conventional protocol). Abnormal ovarian reserve test (antral follicle count (AFC)  $< 5-7$  follicles or anti-Mullerian hormone (AMH)  $< 0.5-1.1$  ng/ml).

**Exclusion Criteria** were ovarian insufficiency due to gonadal dysgenesis and chromosomal abnormalities, Carcinomas or a history of chronic pelvic pain, Current infection, and haemoglobin level lower than 11 g/L or platelet count lower than  $150 \times 10^3/\mu\text{L}$  and patients aged below 20 or above 40.

The study included 20 patients with ovarian failure, divided into two groups: Group A underwent laparoscopic ovarian PRP injection, and Group B underwent laparoscopic ovarian normal saline injection.

**All patients were subjected to** A. Detailed History Taking: A detailed history was taken from each patient, including personal information, infertility period, and primary or secondary infertility, hirsutism, and acne. General medical history, comorbidities, past obstetric history, menstrual history, contraceptive history, medical problems, allergies, and previous operations were also documented. Additionally, family history of infertility or consanguinity was obtained. B. Full Clinical Examination: A comprehensive clinical examination was performed, including a general examination of vital signs and a local examination of the vulva, vagina, and cervix, bimanual examination of uterus and adnexa. C. Routine Laboratory Investigations: Hormonal levels (FSH and LH at day 2-3 of the cycle, estradiol, AMH) were evaluated through laboratory testing. General tests such as CBC, urine

analysis, and random blood sugar were performed when necessary.

D. Preparation of PRP: PRP was prepared according to the manufacturer's guidelines using Ycellbio PRP with a lower concentration (2.5 x 3 times) system. The process followed strict aseptic conditions and temperature regulations (21-24°C). Blood samples were collected, centrifuged, and 20cc of PRP was harvested.

E. Laparoscopic Procedure: The injection technique was conducted under general anaesthesia. 4ml of PRP was injected into the right and left ovary, respectively. After confirming there was no bleeding at the place of needle insertion, the needle was withdrawn, and the wounds were closed. Patients were discharged after postoperative recovery, advised unprotected intercourse, and scheduled for follow-up.

F. Postoperative Follow-up: hormone levels (FSH, LH, AMH, estradiol) were measured every month for six months after treatment to monitor the treatment response.

#### **Statistical analysis:**

The acquired data were updated, categorized, and tabulated with the use of the Statistical programme for Social Science (IBM Corp. IBM SPSS Statistics for Windows, Version 25.0 (Armonk, New York: IBM Corporation, 2005)). Based on the kind of data acquired for each parameter, the appropriate analysis was done on the supplied data. Using the Shapiro-Wilk test, the normality of the data distribution was examined. For regularly distributed numerical data, descriptive statistics such as mean and standard deviation (SD) were computed; for non-normally distributed numerical data, median and range were determined. The frequency and proportion of nonnumerical data were determined. Analytical statistics include the use of Student's T Test and Wilcoxon signed rank test to evaluate the statistical significance of differences between means in two research groups and between dependent

variables within a single group, respectively. A correlation study was performed to evaluate the strength of the relationship between two quantitative variables. The probability of results was assessed using a significance level (p-value) of <0.05 at a 95% confidence interval.

### Results

The current study carried on 20 patients with ovarian failure. They were split into two groups: Group A: Underwent laparoscopic ovarian PRP (Plasma rich plasma) injection. Group B: Underwent laparoscopic ovarian normal saline injection. The mean age of Group A was 28.9±7.37 years, while the mean age of Group B was 29.8±4.59 years with no statically significant (P-value < 0.05).

Hormones were measured before treatment and every month for six months after treatment. According to FSH (follicle stimulating hormone), Group A had a significant reduction in FSH levels over study time from a median of 41.9 IU/L

before treatment to 13.7 IU/L 6 months after treatment (p= 0.005). In contrast, median FSH level in Group B was 45.7 IU/L before treatment and 48.1 IU/L after 6 months of treatment with no significant change (p=0.878). Table 1

In terms of LH (luteinizing hormone), Group A had a significant reduction in LH levels over study time from a median of 19.6 IU/L before treatment to 6.5 IU/L 6 months after treatment (p= 0.006). In contrast, median LH level in Group B was 17.3 IU/L before treatment and 18.0 IU/L after 6 months of treatment with no significant change (p=0.594). Figure 1

Regarding AMH (Anti mullerian hormone), median baseline AMH level in Group A was 0.019 that significantly increased in the 1<sup>st</sup> month to 0.294 (p =0.005) then decreased across 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup>, 5<sup>th</sup> and 6<sup>th</sup> months to 0.252, 0.178, 0.084, 0.039, 0.020 respectively. Th median AMH level in Group B was 0.024 IU/L before treatment and 0.022 IU/L after 6 months of treatment with no significant change (p=0.721). Figure 2

**Table 1:** Comparison between FSH level at different periods in the study groups.

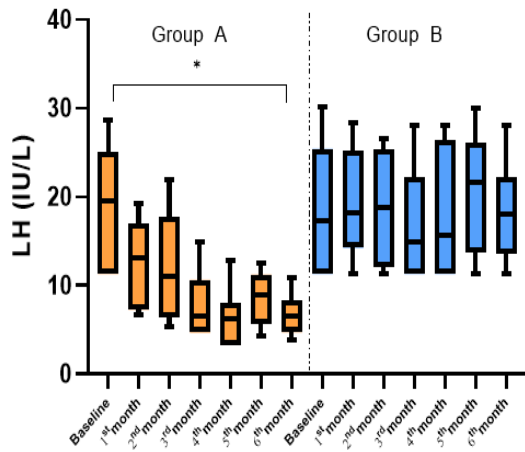
Variable		Group A (n=10)	Group B (n=10)
FSH (IU/L)	<b>Baseline</b>	41.9(19.8-57.0)	45.7(23.4-67.9)
	<b>1st month</b>	25.6(14.7-38.3)	54.7(20.4-68.7)
	<b>2nd month</b>	20.1(10.7-32.3)	47.6(25.4-68.7)
	<b>3rd month</b>	15.5(10.1-39.8)	42.8(20.4-56.6)
	<b>4th month</b>	11.3(8.7-18.3)	37.6(21.5-62.2)
	<b>5th month</b>	12(8.3-21.2)	42.6(20.4-63.3)
	<b>6th month</b>	13.7(6.8-24.0)	48.1(20.4-62.4)
Pretreatment vs Posttreatment	<b>Test</b>	Z= 2.803	Z=0.153
	<b>p</b>	0.005*	0.878

Data represented as Median (IQR); Z= Wilcoxon signed rank test, \*: Significant ≤0.05

**Table 2:** AFC comparison in the study groups before and after treatment.

		Group A (n=10)	Group B (n=10)	Test	p
AFC (n)	<b>Pretreatment</b>	4 (3-5)	4 (3-5)	t=0.717	0.482
	<b>Post treatment</b>	7 (6-8)	4 (3-5)	t=7.099	<0.001*

Data represented as Median (Range); t=Independent t student test; \*: Significant ≤0.05.



**Figure 1:** Box plot comparison of LH level at different periods in the study group.

In terms of E2 (estradiol), Group A had a significant increase in E2 levels over study time from a median of 23.7 pg/ml before treatment to 45.2 pg/ml 6 months after treatment ( $p= 0.007$ ). In contrast, median E2 level in Group B was 21.9 pg/ml before treatment and 19.2 pg/ml after 6 months of treatment with no significant change ( $p=0.285$ ). Figure 3

Before treatment, both groups had a similar AFC with a median of 4 (range 3-5) for both Group A and Group B. After treatment, Group A had a substantial elevation in AFC from a median of 4 to a

median of 7 ( $p<0.001$ ). In contrast, Group B had a similar AFC after treatment with a median of 4 (range 3-5). Table 2

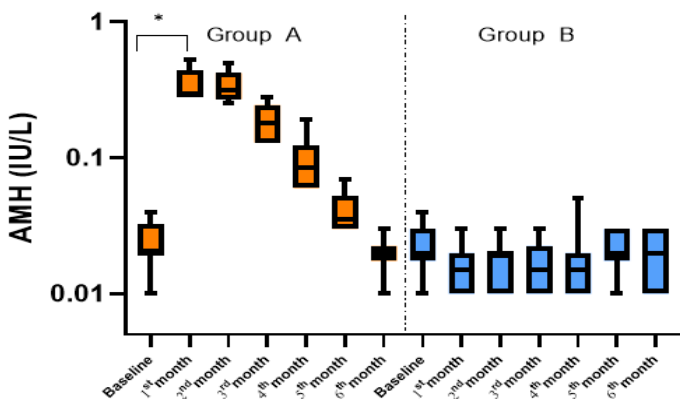
According to the ovulation status of Group A after PRP injection, out of the 10 participants, 3 (30%) ovulated, while 7 (70%) did not ovulate.

Antral follicular count post treatment showed a significant negative correlation with FSH ( $p<0.001$ ) and LH ( $p=0.002$ ). Antral follicular count post treatment revealed a significant positive association with E2 ( $p=0.001$ ) while no significant correlation with age and AMH. Table 3.

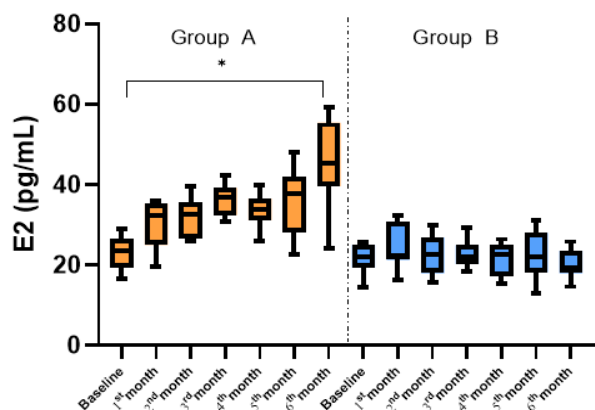
**Table 3:** Correlation between serum AFC with other studied parameters.

	$r_s$	p
Age	-0.166	0.484
FSH	-.787	<0.001*
LH	-.653	0.002*
AMH	-0.011	0.963
E2	0.700	0.001*

rs: Spearman correlation coefficient; \*: Significant  $\leq 0.05$ .



**Figure 2:** Box plot comparison between AMH level at different periods in the study groups.



**Figure 3:** Box plot comparison between E2 level at different periods in the study.

## Discussion

In the current study, the mean age of group A was  $28.9 \pm 7.37$  years, while the mean age of group B was  $29.8 \pm 4.59$  years.

In agreement with our study, some authors conducted a prospective controlled trial to determine if intraovarian injections of autologous PRP may stimulate ovarian rejuvenation and folliculogenesis reactivation in women with early ovarian dysfunction. 50 infertile women with premature ovarian dysfunction were enrolled in the study. They discovered that the average age of the individuals examined was 31.1 years, with a standard deviation of 4.38. The age range varied between 24 and 38 years. Furthermore, the average duration of infertility was 2.66 years, with a standard deviation of 1.33. The range for the duration of infertility was between 1 and 5 years. In terms of BMI, the average value was  $31.11 \text{ kg/m}^2$ , with a standard deviation of 3.48. The BMI ranged from 25 to  $37.6 \text{ kg/m}^2$ . Among the individuals studied, 78% (39) experienced primary infertility, while 22% (11 cases) experienced secondary infertility<sup>(7)</sup>.

In this study, hormones were measured before treatment and monthly for six months after treatment in two groups of patients with poor ovarian response (POR). Group A, receiving autologous PRP injections, showed a significant reduction in both FSH and LH levels over the study period. Additionally, AMH

levels in Group A significantly increased in the 1st month and then gradually decreased, returning to baseline by the 6th month. Conversely, Group B, receiving normal saline injections, did not exhibit significant changes in FSH, LH, or AMH levels over the same period. Moreover, estradiol (E2) levels increased significantly in both groups, with the highest level observed in the 6th month, surpassing the baseline level.

These findings suggest that PRP therapy has a specific impact on FSH levels, resulting in a significant reduction in group A. This reduction in FSH levels indicates an improvement in ovarian function and the potential for enhanced follicular growth. On the other hand, the normal saline group did not experience the same level of improvement in FSH levels, suggesting that the observed effect is likely attributed to the PRP treatment itself<sup>(5)</sup>.

Confirming our findings, a study reported that PRP intervention had significant effects on FSH concentration at  $\alpha = 0.05$  level. Statistically significant increases in normal values of FSH and E2 were observed for months three and four after the PRP intervention for all age groups<sup>(1)</sup>.

In line with our findings, a study observed significant changes in hormone levels over time: FSH decreased, with the lowest level in the 3rd month, LH and E2 also showed decreasing trends. AMH varied across

periods, initially increased and then decreased by the 3rd month. Estradiol levels increased significantly over time ( $P$ -value  $< 0.001$ )<sup>(7)</sup>.

Furthermore, a pioneering study was conducted to compare live birth rates (LBR) between 20 poor responders who received 3-5 mL autologous PRP under transvaginal ultrasonography surveillance and 20 well-matched controls. After  $61 \pm 18$  days, both groups underwent the same low-dose activation protocol with a GnRH antagonist and PRP application. The researchers injected 4 mL PRP into each ovary using 30 mL peripheral blood and observed a significant FSH decrease<sup>(8)</sup>.

A study confirmed our findings, noting significant FSH decrease in the second menstrual cycle post-PRP therapy ( $7.05 \pm 1.43$  UI/ml) compared to pre-treatment levels ( $11.50 \pm 4.05$  UI/ml,  $P < 0.001$ ). FSH levels returned to pre-treatment levels ( $11.28 \pm 3.23$  UI/ml) at six months. LH levels showed similar patterns, with partial recovery at six months ( $6.00 \pm 2.36$  vs. pre-PRP,  $7.25 \pm 1.92$ ). AMH increased significantly post-PRP in both cycles ( $P < 0.05$ ) and slightly decreased by the 6th month ( $0.71 \pm 0.33$ ,  $P < 0.05$ ) but remained higher than pre-PRP ( $0.69 \pm 0.32$ ). Estradiol levels increased approximately 50% on HCG trigger day ( $907.75 \pm 386.56$  vs.  $603.75 \pm 262.24$ ,  $P < 0.001$ )<sup>(9)</sup>.

The findings from the previous study suggest that PRP therapy may have a transient impact on FSH levels. They observed a significant decrease in FSH levels during the second menstrual cycle following PRP therapy compared to the first cycle. This indicates a potential immediate effect of PRP on FSH regulation. However, it is important to note that the FSH levels returned to pre-treatment levels after six months, suggesting that the effect of PRP therapy on FSH may not be sustained in the long term.

However, it is worth noting that the significant reduction in LH levels observed

in group A over the study period aligns with the general understanding of the reciprocal relationship between LH and estradiol. LH is typically involved in triggering ovulation and the production of estradiol, so a reduction in LH levels may correspond to a decrease in estradiol levels.

Parallel to our results, a study on 38 infertile females with low ovarian reserve and reported that intraovarian PRP injections led to significant and sustained decreases in FSH and LH levels throughout the study ( $p < 0.0007$ – $0.00004$ ). AMH levels improved dramatically, reaching 1.1 ng/ml from 0.08 ng/ml pre-treatment, aiding successful pregnancy or egg retrieval. Estradiol levels steadily rose from 1st to 6th month post-PRP, slightly declining at 12 months. The most significant estradiol levels were at 6th and 12th months compared to pre-rejuvenation levels ( $p < 0.0003$ ;  $p < 0.00005$ ), attributed to PRP's regulatory and immunomodulatory effects<sup>(10)</sup>.

Supporting our findings, a study investigated PRP's ovarian rejuvenation efficacy. They studied 253 women (age 22–56) across five groups, evaluating FSH, LH, E2, and AMH levels after PRP infusion. They found after the two-month follow-up, the majority of the participants presented improvement in their hormonal profiles<sup>(3)</sup>.

A recent prospective cohort study that was conducted to see if intraovarian injection of PRP change ovarian function in patients with extremely low functional ovarian reserve (LFOR) who, otherwise, would likely only have a chance of pregnancy through third-party oocyte donation. In their study, 80 consecutive patients at ages 28-54 with LFOR, defined by anti-Müllerian hormone  $< 1.1$  ng/ml, FSH  $> 12$  mIU/ml or at least one prior IVF cycle with  $\leq 3$  oocytes within 1 year. The women were followed for 1 year after an intraovarian PRP procedure. PRP (1.5 ml)

was injected into the cortex of ovaries with an average of 12 injections per ovary<sup>(11)</sup>.

The study participants were followed every 3 days for 2 weeks after PRP treatment with estradiol and FSH measurements and vaginal ultrasound to observe follicle growth and thereafter followed weekly. Beginning 1 month after their PRP treatment, participants underwent one or more cycles of ovarian stimulation for IVF. Outcome measures were endocrine response, and numbers of oocytes and embryos produced in response to maximal gonadotropin stimulation before and after PRP treatment. They found no clinically significant effects of PRP treatment on ovarian function were observed over 1 year of follow-up<sup>(11)</sup>.

Furthermore, a clinical trial was conducted with 35 women having poor ovarian reserve (POR) and mean age  $40.68 \pm 0.34$ . They administered a single dose intraovarian autologous PRP injection and assessed oocyte count, antral follicles, estradiol, AMH, FSH, LH, and FSH/LH ratio before and after treatment. Serum FSH ( $12.2 \pm 0.31$  to  $12.51 \pm 0.28$ ) and LH ( $13.00 \pm 0.25$  to  $13.14 \pm 0.26$ ) levels didn't significantly change post-PRP. AMH ( $0.38 \pm 0.039$ ) remained unchanged compared to pre-treatment ( $0.39 \pm 0.04$ ). However, they observed a substantial increase in estradiol post-PRP ( $404.1 \pm 16.76$ ) compared to before ( $237.7 \pm 13.14$ ,  $P=0.0003$ )<sup>(12)</sup>.

Further, a study demonstrated that intra-ovarian injection of autologous PRP in the women with primary ovarian insufficiency had no significant effect on the FSH levels, and also, associated with minimal improvement in the AMH levels<sup>(13)</sup>. Although, a study did not observe a significant difference in the hormonal (LH and FSH) profile of women with POR or primary ovarian insufficiency after PRP injection<sup>(14)</sup>.

The results of our study indicate that PRP therapy had a significant impact on the antral follicle count (AFC) and ovulation status in poor ovarian reserve (POR) patients. Group A, which received PRP

injections, demonstrated a significant increase in AFC from a median of 4 to a median of 7 after treatment, while Group B, which received saline injections, showed no significant change in AFC. This finding is consistent with previous studies that have reported the beneficial effects of PRP therapy on AFC.

Consistently, a study) found that PRP treatment resulted in higher AFC, higher serum AMH, lower serum FSH, and a higher number of mature oocytes and cleavage and blastocyst stage embryos<sup>(15)</sup>.

A study also found a significant increase in AFC following PRP therapy in POR patients. They reported that AFC significantly improved from baseline after PRP treatment, supporting the notion that PRP can enhance follicular development and potentially improve fertility outcomes<sup>(8)</sup>.

In terms of ovulation status, our study revealed that 30% of participants in Group A ovulated after PRP injection, while 70% did not ovulate. Although the ovulation rate was modest, it is noteworthy that PRP therapy showed the potential to induce ovulation in a subset of patients. This finding aligns with the findings of other studies. A study by a study observed improved ovulation rates in infertile females with low ovarian reserve who received PRP intraovarian injections. These studies collectively suggest that PRP therapy may have a positive impact on ovulation in POR patients<sup>(10)</sup>.

Additionally, our study found that post-treatment AFC showed a significant negative correlation with FSH and LH levels. This implies that higher AFC was associated with lower FSH and LH levels, indicating improved ovarian function and reduced inhibition of follicular development. Conversely, post-treatment AFC showed a significant positive correlation with estradiol (E2) levels, indicating that higher AFC was associated with higher E2 levels, which is indicative of better ovarian function and follicular growth.



These correlations are in line with previous research, such as the findings of a study reported similar correlations between AFC, FSH, LH, and E2 levels in POR patients undergoing PRP therapy<sup>(9)</sup>.

## Conclusion

In conclusion, PRP therapy leads to a substantial reduction in follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels, indicating improved ovarian function and enhanced follicular development. Additionally, PRP treatment results in a significant increase in estradiol (E2) levels, reflecting an improvement in ovarian hormone production. Antral follicle count (AFC), a marker of ovarian reserve and follicular growth, significantly increases after PRP therapy, suggesting enhanced follicular development. However, the effects on anti-Mullerian hormone (AMH) levels are transient, returning to baseline by the sixth month after the treatment.

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## Author contribution

Authors contributed equally in the study.

## Conflicts of interest

No conflicts of interest

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