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Differences in Anti-Müllerian Hormone Levels Before and After Platelet-Rich Plasma–Based Ovarian Rejuvenation in Patients with Premature Ovarian Insufficiency

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Abstract

Premature Ovarian Insufficiency (POI) is defined as a decline in ovarian function before the age of 40, with an estimated prevalence of approximately 3.7%, particularly higher in countries with low to medium Human Development Index (HDI). POI can negatively affect both physical and psychological health in women. Anti-Müllerian Hormone (AMH) is widely recognized as a biomarker to assess ovarian reserve. Recently, Platelet-Rich Plasma (PRP) therapy has been investigated as a novel approach to enhance AMH levels and rejuvenate ovarian function in POI patients. To evaluate the effect of PRP-based ovarian rejuvenation therapy on AMH levels in women with POI. This retrospective observational analytic study involved 33 women diagnosed with POI who underwent PRP ovarian rejuvenation therapy at Bali Royal Hospital between 2022 and 2024. Total sampling was employed, and secondary data were obtained from patient medical records. Data were analyzed using univariate and bivariate methods, specifically paired-sample t-tests. The study included 33 women with a mean age of 35.62 ± 5.68 years. The mean baseline AMH level was 0.53 ± 0.34 ng/mL, which increased to 0.66 ± 0.46 ng/mL three months after PRP therapy. Statistical analysis demonstrated a significant increase in AMH levels following PRP treatment ($p \leq 0.05$). A statistically significant difference in AMH levels was observed before and three months after PRP-based ovarian rejuvenation therapy in women with POI treated at Bali Royal Hospital during the 2022–2024 period. These findings indicate that PRP may represent a promising adjunctive therapy for improving ovarian reserve in patients with POI.

Introduction

Therapeutic modalities for patients with infertility are increasingly developing (Devroey et al., 2009; Fernandez, H., & Gervaise, 2004). One modality that is currently popular is ovarian platelet-rich plasma (PRP) injection. PRP is an autologous blood product that has a platelet concentration three to five times higher than normal (Orisaka et al., 2021). PRP provides a mixture of proangiogenic, proliferative, and proinflammatory factors that can stimulate de novo oogenesis and/or follicular maturation. Several studies have shown that PRP treatment may be an option for patients with Premature Ovarian Insufficiency (POI) (Orisaka et al., 2021).

POI is a condition in which ovarian function begins to decline or does not function normally in women before the age of 40 (Ishizuka, 2021; Kirshenbaum & Orvieto, 2019; Eshre et al., 2016). This condition is characterized by prolonged amenorrhea. POI occurs in about 1% of the population and is idiopathic. About 4-10% of women with POI can conceive naturally,

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while about 20% are successful with ovulation induction. The prevalence of POI is reported to be higher in countries with a medium or low Human Development Index, at around 3.7% (Golezar et al., 2019).

Risk factors for POI are associated with several factors such as genetics, metabolism, autoimmunity, iatrogenic factors, lifestyle, and infections, especially those caused by viruses, which cause a reduction in the number of remaining ovarian follicles and ovarian sex hormone deficiency..4 Ovulation induction for women with POI is performed to create ovulation conditions by producing one mature follicle that will eventually ovulate to obtain multiple follicles, thereby increasing the pregnancy rate (Lin et al., 2021; Podfigurna-Stopa et al., 2016).

One of the hormones that acts as a marker for detecting POI is Anti-Mullerian Hormone (AMH), Follicle Stimulating Hormone (FSH), and Antral Follicle Count (AFC).1,2 Anti-Mullerian Hormone (AMH) is a form of glycoprotein transformation from transforming growth factor-B (TGF-B). AMH is produced by the granulosa cells in preantral follicles and small antral follicles of the ovarian follicles. AMH levels peak during the late follicular phase.

This hormone undergoes changes earlier than the increase in basal FSH when ovarian reserve begins to decline and does not undergo significant changes during the menstrual cycle, making it the most widely used marker for detecting POI (Barakat et al., 2023). To date, PRP as a rejuvenation modality is still widely researched, although it remains controversial, prompting the author to conduct research on the ability of PRP to increase ovarian reserve.

Methods

The research design used in this study was a retrospective, observational analysis design which aims at assessing changes in the levels of the Anti–Mullerian Hormone (AMH) levels before and after the treatment of Platelet-Rich Plasma (PRP)-based ovarian rejuvenation therapy in patients diagnosed with Premature Ovarian Insufficiency (POI). The study was based on the Bali Royal Hospital, Denpasar, and it was made on the basis of secondary data obtained through medical records that were carried out in January 2022 and December 2024.

The target population of the study was all the female patients with clinical diagnosis of POI who underwent intraovarian PRP therapy within the period of study. A total sampling approach was followed to capture all the eligible subjects who fit into the inclusion criteria in order to minimize selection bias and give representativeness to the available population.

The inclusion requirements were women who had a clinical diagnosis of POI and were aged between 20 and 50 years with a full medical history of baseline and follow up AMH measurements. The exclusion criteria were patients who had complete records, patients who did not have post-treatment laboratory results, or patients who had undergone other ovarian procedures within the observation time that might confound hormonal results.

The systematic review of hospital medical records was used to collect data, which was carried out through the use of a standardized data extraction form. The variables that were extracted were patient age, diagnosis of POI, the baseline AMH level before PRP therapy, and the measurement of the AMH level three months after the intervention. The laboratory findings were all through the certified clinical laboratory in the hospital, which followed the standardized testing protocol to guarantee reliability of measurements.

The PRP intervention was implemented based on the clinical protocol of the hospital. Each patient donated autologous blood which was harvested and centrifuged twice using a double centrifugation technique to create platelet-rich plasma. Gynecologists trained on trans -vaginal ultrasound then injected the prepared PRP into the ovarian tissue. This was done in a sterile environment to ensure patient safety and consistency of the procedure.

Appropriate software was used in the statistical analysis. Descriptive statistics were used to provide a description of the characteristics and baseline variables of the participants, and the mean values and standard deviations were described. Appropriate tests were used to test the normality of the data distribution. Since there was normal data distribution, the inferential analysis included a paired-sample t test that compared the AMH levels between pre and post PRP.

The statistical level of significance was established as $p < 0.05$ and was used to ascertain whether the differences that exist in AMH levels were statistically significant.

Results and Discussion

Based on the collection of medical records of women with POI who underwent PRP ovarian rejuvenation at Bali Royal Hospital, there were 34 subjects who met the inclusion criteria. The average age of women with POI who underwent PRP ovarian rejuvenation was 35.62 ± 5.68. Before undergoing PRP, the average AMH level of respondents was 0.53 ± 0.34, and after three months of ovarian rejuvenation with PRP, there was an increase in AMH levels, which became 0.66 ± 0.46.

Table 1. Patient Characteristics

Variable	N (%)	Mean
Age		35.62 ± 5.68
AMH level		
Pre-Treatment AMH Level		0.53 ± 0.34
AMH Level Post-Treatment		0.66 ± 0.46

The collected data was then tested for normality and found to have a p value > 0.05, indicating that the data was normally distributed. The comparison of AMH levels before and after PRP ovarian rejuvenation in women with POI was then analyzed using a paired sample test.

Table 2. Comparison of AMH Levels Before and After PRP Ovarian Rejuvenation Treatment in Women with POI

Treatment	Mean ± SD	N	p value
AMH pre-treatment	0.53 ± 0.34	34	0.031
AMH post-treatment	0.66 ± 0.46	34	

Based on Table 2, the average AMH level in women with POI before PRP ovarian rejuvenation treatment was 0.53 ± 0.34, and the AMH level after treatment was 0.66 ± 0.46. Statistical testing yielded a p-value of 0.031 (p-value < 0.05), indicating a statistically significant difference in AMH levels before and after PRP treatment in women with POI.

Premature ovarian insufficiency (POI) is a condition in which ovarian follicles thin and cease to function normally in both the reproductive and endocrine organs of women under the age of 40. The exact mechanism of POI is still unknown. Approximately 90% of diagnosed cases of spontaneous POI have no underlying etiology. Other factors that contribute to the development of POI include iatrogenic factors (oophorectomy, chemotherapy, or radiation), chromosomal abnormalities (Turner syndrome, which causes premature oocyte apoptosis in the uterus and accelerated oocyte depletion before the age of 10), and autoimmune diseases (adrenal insufficiency, hypothyroidism, systemic lupus erythematosus). Genetic mutations and autosomal recessive diseases such as galactosemia (reduction of galactose-1-phosphate), ataxia-telangiectasia (ATM gene), and blepharophimosis-ptosis-epicanthus-inversus syndrome (BPES, mutation in FOXL2) have not been defined in POI. Environmental exposures such as smoking, nicotine, and various substances contribute to POI by binding to receptors on ovarian

granulosa cells, thereby activating proapoptotic genes and inhibiting aromatase , which then leads to a decrease in circulating estradiol (Kapoor, 2023; Sopiarz & Sparzak, 2025).

POI affects approximately 1 to 2% of women under the age of 40 and even fewer women under the age of 30 (0.1%) (Chon et al., 2021). In this study, the average age of women with POI was 35.62 ± 5.68. The results of this study are consistent with a previous study conducted in Finland, where the highest incidence occurred in the 35-39 age group, ranging from 73.8/100,000 women in 1993-1997 to 39.9/10,000 women in 2013-2017. The average age at POI diagnosis was highest in 1993-1997 (33.5 years) and lowest in 2013-2017 (31.4 years) (Silvén et al., 2022). The incidence rate of POI varies with age, with a ratio of 1:100 cases at age 40, 1:250 cases at age 35, 1:1000 cases at age 30, and 1:10,000 cases at age 18-25 (Allshouse et al., 2015). When evaluating patients with suspected POI, it is important to obtain a comprehensive and complete history, including current medical history, medical comorbidities, gynecological history, obstetric history, and social factors, including potential stress triggers (Sopiarz & Sparzak, 2025). A diagnosis of POI can be established if there is an increase in FSH at two time points at least four to six weeks apart in women under 40 years of age after more than four months of amenorrhea or irregular menstruation and secondary causes of amenorrhea have been ruled out (Panay et al., 2024).

In the hypergonadotropic-hypogonadal hormone profile examination, a decrease in estrogen (E2) levels of < 20 pg/ml, an increase in gonadotropin levels (FSH > 20 IU/L), low AMH levels - <0.5 ng/ml (<1 ng/ml), and low inhibin B levels are found (Jankowska, 2017). The most commonly used diagnostic threshold for FSH levels is > 40 IU/L. Meanwhile, the National Institute for Health and Care Excellence guidelines suggest an FSH level of > 30 IU/L, and the European Society of Human Reproduction and Embryology guidelines suggest a lower threshold of > 25 IU/L. In patients who are still menstruating, testing should be performed on the second to third day of the menstrual cycle (Kirshenbaum & Orvieto, 2019). Low estrogen (E2) levels are caused by ovarian dysfunction in cases where the feedback mechanism stimulates the pituitary gland to release gonadotropin hormones (high FSH levels).

AMH levels decrease with age, which is used as a marker for decreased fertility, including POF. Inhibin B serves to assess ovarian reserve, but its measurement depends on the phase of the cycle because it is produced by early antral follicular granulosa cells, especially in the follicular phase of the menstrual cycle. In the early follicular phase, inhibin B levels indicate the number and quality of ovarian follicles, so in POF patients, there is a decrease in inhibin B levels (Jankowska, 2017). Patient-oriented strategies encompassing individualized oocyte number (POSEIDON) classify patients into four groups based on age, ovarian reserve markers (AMH, AFC), and the number of oocytes retrieved during a conventional ovarian stimulation (OS) cycle. Patients who meet the POSEIDON criteria have a poor prognosis in ART due to a reduced number of oocytes, which will limit the number of embryos produced (Esteves et al., 2019).

Table 3. POSEIDON Classification

Group	Description
POSEIDON 1	Patients under 35 years of age with normal ovarian reserve markers (AMH > 1.2 ng/mL, AFC > 5) and *unexpected poor ovarian response* (POR) Subgroup 1a: < 4 *retrieved oocytes* in conventional COS during ART/IVF cycles Subgroup 1b: 4–9 *retrieved oocytes* in conventional COS during ART/IVF cycles

POSEIDON 2	Patients over 35 years of age with normal ovarian reserve markers (AMH > 1.2 ng/mL, AFC > 5) and *unexpected poor ovarian response* (POR) Subgroup 2a: < 4 *retrieved oocytes* in conventional COS in ART/IVF cycles Subgroup 2b: 4-0 *retrieved oocytes* in conventional COS in ART/IVF cycles
POSEIDON 3	Patients under 35 years of age with poor ovarian reserve (AMH < 1.2 ng/mL, AFC <5)
POSEIDON 4	Patients over 35 years of age with poor ovarian reserve (AMH < 1.2 ng/mL, AFC < 5)

Anti-Mullerian Hormone (AMH) is a peptide growth factor produced by granulosa cells in preantral follicles and small antral follicles of the ovarian follicles. AMH plays a role in the growth and differentiation of secondary ovarian granulosa cells, preantral and early antral follicles, and can be used as an early indicator of menopausal transition. The release of AMH from ovarian granulosa cells is proportional to the number of developing follicles in the ovary, making it a marker for ovarian aging.5 A poor response is observed at serum AMH levels < 1 ng/ml, a normal response at 1-4 ng/ml, and a good response at > 4 ng/ml (Vijay et al., 2022). Measuring AMH to assess ovarian reserve has many advantages over other markers. Changes in serum AMH levels occur earlier than increases in basal FSH when ovarian reserve begins to decline. Additionally, AMH levels do not undergo significant changes during the menstrual cycle compared to FSH (Lin et al., 2021).

Platelet-rich plasma (PRP) is an autologous blood product that has a platelet concentration three to five times higher than normal. In normal individuals, the platelet count is 1.5-4.4 x 105/µL. The platelet count in PRP cannot be determined, but for therapeutic effectiveness, a platelet concentration 4-5 times higher is required (Zadehmodarres et al., 2017). Based on cell content and fibrin architecture, PRP is divided into four parts: pure platelet-rich plasma (P-PRP) without leukocytes with low-density fibrin network after activation, leucocyte-PRP (L-PRP) containing leukocytes with low-density fibrin tissue ( ) after activation, pure platelet-rich fibrin (P-PRF) without leukocytes with high-density fibrin tissue, and leucocyte and platelet-rich fibrin (L-PRF) containing leukocytes and high-density fibrin tissue. PRP is made by taking a 30 cc sample of the patient's venous blood during treatment, which can produce about 3-5 cc of PRP. When drawing venous blood, an anticoagulant such as citrate dextrose A must be added to prevent platelet activation. Whole blood (WB) is placed in a tube containing anticoagulant at a volume ratio of 10:1. Next, centrifugation is performed twice. The first centrifugation is performed at a constant speed to separate the red blood cells into three fractions: the upper layer containing platelets and WBCs, a thin intermediate layer (buffy coat) rich in white blood cells, and the lower layer consisting of red blood cells. During the second centrifugation, the platelets suspended in the final plasma volume are concentrated. P-PRP is produced from the upper layer and superficial buffy coat. L-PRP is produced from the transfer of the entire buffy coat layer and some red blood cells. Before being applied to the target location, a platelet activator such as 10% CaCl2 laurate needs to be added at a volume ratio of 6:1 or 10:1 to stimulate platelet degranulation and initiate the clotting process (Gutiérrez et al., 2017).

After platelets in PRP are activated, α-granules release biologically active factors including platelet-derived growth factor (PDGF), transforming growth factors-β (TGFs-β), insulin-like growth factor-1 (IGF-1), vascular endothelial growth factors (VEGFs), epidermal growth factors (EGFs), fibroblast growth factors (FGFs), hepatocyte growth factor (HGF), stem cell factor (SCF), connective tissue growth factor (CTGF), neurotropin, granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), growth hormone (GH), and proinflammatory cytokines that can induce accelerated

angiogenesis, cell migration, differentiation, and proliferation. The presence of these growth factors modulates folliculogenesis in the ovary (Ferrari et al., 2021). In the early stages, these cells help increase oocyte survival and trigger the formation of primordial follicles through smad2/smad3 signaling via the kit ligand/c-Kit pathway. Primordial follicles then transition to primary follicles regulated by BMP7 and BMP4 (Vo et al., 2021). In the early stages of folliculogenesis, bFGF, HGF, and SCF induce the activation of primordial follicles through folliculogenesis. During folliculogenesis, there is an increase in the number of blood vessels stimulated by angiogenic factors. PRP contains several factors such as VEGFs, FGFs, and IGFs that can activate ovarian neovascularization, increase follicle growth and oocyte quality (Vo et al., 2021). VEGF is involved in neovascularization through significant endothelial chemokines and mitogenic effects and VEGF-mediated ovarian blood flow, which is an important factor in impaired folliculogenesis. Platelets release various cytokines in response to activation. Cytokine signaling is involved in the reciprocal relationship between oocyte, granulosa, and thecal cells, with dysfunction in this ecosystem leading to deficiencies in follicular maturation, ovulation, and luteinization (Fraidakis et al., 2023).

A number of cytokines that regulate follicular development are released by platelets through alpha and dense granule secretion during platelet activation. PRP provides a mixture of proangiogenic, proliferative, and pro al factors that can stimulate de novo oogenesis and/or follicular maturation. PRP acts proangiogenically through the action of cytokines released by platelets, including VEGF. Primordial follicles depend on stromal blood vessels but are progressively enclosed in thecal capillary tissue during maturation, reflected by increased VEGF expression that persists until corpus luteum formation. Heterozygous knockdown of the hypoxia-response element in the VEGFA promoter or VEGFR antagonism in mouse ovaries causes vascular malformations that result in poor ovarian response to stimulation, indicating the role of VEGF in follicle development and the importance of overall regulated vascularization in follicle development. In addition, the main component of platelet release, PDGF, is also involved in regulating blood vessel formation and maturation (Feng et al., 2017).

Intraovarian PRP administration can cause a progressive increase in AMH and estradiol levels and a decrease in FSH and LH levels. Based on the results of the study, the AMH level before the procedure was 0.53 ± 0.34, while the AMH level after the procedure was 0.66 ± 0.46. Based on the analysis conducted, there was a statistically significant difference in AMH levels before and after the PRP ovarian rejuvenation procedure in infertile patients with POI (p value < 0.05). The growth factors contained in PRP have an impact on tissue regeneration and wound healing. PDGF and VEGF play a role in cell proliferation and migration, while TGF plays a role in angiogenesis and the process of neo-angiogenesis. The concentration of PDGF, VEGF, and TGF through autologous PRP administration can activate endothelial cells to stimulate the neo-angiogenesis process, which can improve the ovarian environment, thereby supporting the growth of secondary pre-antral follicles that then produce ovulatory antral follicles (Ferrari et al., 2021).

The study conducted by Pantos et al analyzed three patients diagnosed with menopause with elevated FSH levels accompanied by amenorrhea for at least one year. FSH, LH, R2, and AMH levels were measured on an unspecified day prior to the procedure, and evaluation after PRP treatment was performed on the second day of the next menstrual cycle. Injections and diffusion into the ovarian stroma were performed using a multifocal intramedullary procedure guided by transvaginal ultrasound. Once identified, the needle was inserted, penetrating the ovarian tissue with resistance. Before the injection procedure, the needle guide was aligned with the ovary to ensure that surrounding structures such as blood vessels were not disturbed. The injection procedure duration ranged from 5 to 7 minutes. Patients are asked to remain in a supine position for 10-15 minutes. Follow-up on the three cases was conducted during the first menstrual cycle after the PRP procedure. In this study, there was a decrease in FSH levels and

an increase in AMH in all cases, restoration of the menstrual cycle, and pregnancy through natural conception 2-6 months after PRP (Pantos et al., 2019).

The study conducted by Cakiroglu et al involved 311 women with POI who received transvaginal PRP injections guided by ultrasound. The injections were administered 10 days after the end of menstruation. The injections were administered under the ovarian cortex into the subcortical and stromal areas at a dose of 2-4 ml per ovary. After the injections, follow-up was conducted for 6 weeks to observe the possibility of spontaneous pregnancy or menstruation. The study found a significant increase in AFC and serum AMH, and 7% of patients experienced spontaneous conception. Overall, 41% of patients undergoing stimulation achieved at least one embryo (Cakiroglu et al., 2022). Abdulla et al investigated the effectiveness of transvaginal PRP injections in women with poor ovarian reserve. In this study, subjects received 1.25 ml injections into each ovary for 10-15 minutes. After four weeks of PRP injections, there was a decrease in FSH levels, an increase in AMH levels, followed by an increase in AFC and mean ovarian volume (Abdullah et al., 2019).

Research conducted by Sills et al found that self-activated intra-ovarian PRP injections can increase serum AMH hormone levels in patients with ovarian dysfunction. The effect of PRP in the ovary is to increase angiogenesis through cytokines released by platelets such as VEGF. Ono et al stated that the positive effect of PRP on the ovary is through sphingosine 1-phosphate (S1P), where S1P can increase follicle maturation and increase the expression of CCN2, which is a factor in connective tissue growth that promotes follicle maturation. In addition, PRP injections can increase in vitro growth and viability of pre-antral follicles (Sills & Wood, 2019). A study conducted by Li et al. involving 793 patients investigated the correlation between intraovarian PRP injections and improved POR. The study found that intraovarian PRP injections can increase AMH levels, increase the number of AFCs, oocytes, and embryos. After 2 months of PRP injections, the ovaries showed better results (Li et al., 2023). A study by PACU et al. showed that after 6 months of PRP injections, several indicators returned to the same levels as before PRP, indicating that a two-month PRP injection intervention was more effective and helped reproductive function. A PRP injection dose of $\geq$ 4 ml in each ovary showed a significant increase in AFC, oocyte count, and embryo count compared to a dose of $<$ 4 ml. However, no significant difference was found between PRP injection doses $<$ 4 ml and $\geq$ 4 ml in patients' AMH ($p > 0.05$), with overall results showing that AMH improved after treatment (Pacu et al., 2021).

Conclusion

This study demonstrates that Platelet Rich Plasma based ovarian rejuvenation therapy is associated with a statistically significant increase in Anti Müllerian Hormone levels in women diagnosed with Premature Ovarian Insufficiency at Bali Royal Hospital during the 2022 to 2024 period. The observed improvement in AMH levels three months after intervention suggests that PRP may contribute to the enhancement of ovarian reserve and reflect a potential restoration of ovarian functional activity in selected patients.

Although the findings indicate a positive therapeutic effect, this study is limited by its retrospective design, relatively small sample size, and reliance on secondary medical record data. These factors may restrict the generalizability of the results and preclude causal inference. Therefore, further prospective studies with larger populations, longer follow up periods, and controlled designs are required to confirm the long term efficacy, safety, and reproductive outcomes of PRP based ovarian rejuvenation. Future research should also explore standardized PRP preparation protocols and patient selection criteria to optimize clinical applications in reproductive medicine.

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