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Effects of Intraovarian Platelet-Rich Plasma on Ovarian Reserve and IVF Outcomes

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Article

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Effects of Intraovarian Platelet-Rich Plasma on Ovarian Reserve and IVF Outcomes

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Abstract

Background *Diminished ovarian reserve (DOR)* is a major challenge in infertility management, and regenerative approaches such as intraovarian platelet-rich plasma (PRP) have recently gained attention as potential therapeutic options. This study aimed to evaluate the effect of intraovarian PRP injection on ovarian reserve markers in women with DOR.

Methods In this clinical trial, 15 infertile women with confirmed DOR referred to the infertility center of Imam Khomeini Hospital (Sari, Iran) were enrolled. Baseline ovarian reserve parameters—including antral follicle count (AFC), antimüllerian hormone (AMH), follicle-stimulating hormone (FSH), and estradiol (E2)—were assessed on day 3 of the menstrual cycle. All participants underwent a GnRH antagonist protocol, and on the day of oocyte retrieval, 4 ml of autologous calcium-gluconate–activated PRP (1 ml injected into the ovarian stroma and 1 ml into the cortex of each ovary) was administered. Two weeks after PRP injection, AMH, FSH, E2, and AFC were evaluated.

Results Following PRP administration, AMH levels showed an increase (0.54 ± 0.29 to 0.64 ± 0.55 ng/ml). Although FSH, E2, and AFC demonstrated changes after treatment, these differences were not statistically significant ($P > 0.05$). Among the participants, one pregnancy resulted in miscarriage and one in a live birth.

Conclusions Intraovarian PRP injection may serve as a supportive therapeutic option for infertile women with DOR, particularly those of advanced reproductive age or those wishing to pursue IVF using autologous oocytes. However, larger controlled studies are required to clarify its clinical efficacy, long-term benefits, and underlying mechanisms in ovarian tissue regeneration.

Trial registration Present study registered at Iranian Registry of Clinical Trials: IRCT20160815029374N7, on 2019-06-20

Keywords Infertility, Platelet Rich Plasma, Diminished Ovarian Reserve, In Vitro Fertilization, Anti mullerian Hormone.

Background

Diminished ovarian reserve (DOR) is a major contributor to age-related infertility, as both the quantity and functional competence of oocytes decline progressively with advancing reproductive age. This reduction is influenced by intrinsic ovarian aging, mitochondrial dysfunction, and alterations in the neuroendocrine environment, all of which contribute to impaired reproductive potential (1,2). The ovarian surface epithelium undergoes repeated micro-injury during ovulatory cycles, and accumulating evidence suggests that ovarian stem or progenitor cells may participate in post-ovulatory tissue repair and follicular renewal (3,4).

In recent years, intraovarian platelet-rich plasma (PRP) has gained attention as a potential regenerative approach for women with DOR. PRP, first introduced in the 1970s, contains a high concentration of autologous platelets enriched with growth factors and cytokines that regulate cellular proliferation, angiogenesis, extracellular matrix remodeling, and tissue repair (5–7). Its autologous nature, ease of preparation, and favorable safety profile have facilitated its application across multiple medical fields, including reproductive medicine (8,9). Several studies have demonstrated that PRP may enhance endometrial receptivity, improve implantation rates, and modulate ovarian function (10–13).

The first clinical application of intraovarian PRP for ovarian rejuvenation was reported by Pantos et al., who observed improved ovarian activity in perimenopausal women (14). Subsequent studies have shown increases in antimüllerian hormone (AMH) levels and variable improvements in ovarian reserve markers following PRP administration (15–18). However, despite growing clinical interest, the underlying mechanisms of PRP action within ovarian tissue remain poorly understood, and reported outcomes across studies are heterogeneous (19).

Given the increasing demand for fertility preservation and the desire of many women to conceive using their own gametes at older reproductive ages, further investigation into regenerative interventions is warranted. Therefore, the present study aimed to evaluate the effects of intraovarian PRP injection on ovarian reserve parameters in infertile women diagnosed with DOR.

Materials and methods

Ethical approval

Present study was not blinding clinical trial, registered at Iranian Registry of Clinical Trials: IRCT20160815029374N7, This study was registered on 2019-06-20, conducted in Infertility Center, Imam Khomeini Hospital, Sari, Iran and approved by Ethical Committee at Mazandaran University of Medical Sciences: IR.MAZUMS.IMAMHOSPITAL.REC.1398.007. Also written informed consent was obtained from all patients. The study adhered to the ethical principles outlined in the Declaration of Helsinki (2013 revision). To ensure participant confidentiality and privacy, all collected data were anonymized and securely stored.

Study population

The present study was conducted between April and August 2019 and included a cohort of 15 infertile women diagnosed with diminished ovarian reserve who were referred to the Infertility Center of Imam Khomeini Hospital in Sari. Eligible participants were women with a history of infertility exceeding one-year, suboptimal oocyte or embryo quality in previous IVF cycles, and biochemical evidence of reduced ovarian reserve, defined as anti-Müllerian hormone (AMH) levels ≤1.1 ng/mL and antral follicle count (AFC) <5. In addition, participants exhibited oligomenorrhea or amenorrhea for a minimum duration of four months [20].

Exclusion criteria were rigorously applied to minimize confounding factors and included hematologic disorders such as thrombocytopenia; immunologic abnormalities including antiphospholipid syndrome and thrombophilia; any history of malignancy; current or previous IgA deficiency; prior pelvic inflammatory disease; chronic pelvic pain; ovarian insufficiency secondary to sex chromosome abnormalities; previous abdominal or pelvic surgery associated with adhesions; endometriosis; psychiatric illness; substance abuse or dependence; use of anticoagulant medications; and infertility attributed solely to male factor.

This stringent selection framework ensured a homogenous study population and enhanced the internal validity of the investigation.

Preparation and activation PRP

Preparation and activation of PRP were performed using the standardized PRP kit manufactured by Aria Gene Company. A total of 40 mL of peripheral venous blood was collected from each participant and transferred into two sterile tubes preloaded with a thixotropic separation gel and sodium citrate anticoagulant. The samples were immediately centrifuged at 1,500 g for 5 minutes at room temperature. Following centrifugation, erythrocytes were retained beneath the gel barrier, while platelet-rich plasma and the buffy coat accumulated above it, allowing efficient separation of plasma components.

Based on the minimum requirement of 1 mL PRP for the stromal region and 1 mL for the cortical region of each ovary, a total volume of 4 mL PRP was prepared for intraovarian administration. Activation was achieved using

10% calcium gluconate, prepared by mixing 0.1 mL calcium gluconate with 0.9 mL PRP in an insulin syringe, as previously described [9,21]. The activated PRP was then divided equally into two syringes and allowed to reach room temperature prior to injection. Each syringe was subsequently connected to a 35-cm, 17-gauge single-lumen needle suitable for transvaginal ovarian access.

Gonadotropin Stimulation Protocol

Baseline serum levels of AMH, FSH, and estradiol were assessed prior to initiating controlled ovarian stimulation. All participants underwent a standardized GnRH antagonist protocol. Beginning on day 3 of the menstrual cycle, patients received 225–400 IU/day of recombinant FSH (Cinal-F; Sina Gene, Iran), with the initial dose individualized according to age and ovarian reserve parameters (22). After five days of stimulation, serial transvaginal ultrasonography was performed to monitor follicular development.

When the leading follicle reached 12–14 mm, daily administration of 0.25 mg Cetrorelix (Cetrotide; Merck Serono, Geneva, Switzerland) was initiated to prevent premature LH surge (23). Gonadotropin dosage was subsequently adjusted based on follicular growth kinetics and ovarian response (24). Final oocyte maturation was triggered with 250 μg recombinant hCG (Ovitrelle; Merck Serono, Geneva, Switzerland) once at least two follicles reached ≥17 mm (25). Oocyte retrieval was performed 34–36 hours later under intravenous anesthesia.

Intraovarian Injection

On the morning of oocyte retrieval, patients received conscious analgesia. Activated PRP—prepared less than 30 minutes prior—was injected transvaginal under continuous ultrasound guidance. Using a single-lumen 17-gauge, 35-cm needle, 1 mL of PRP was administered into the ovarian stroma and 1 mL into the cortex of each ovary (26–28). The needle was advanced directly into the ovarian parenchyma without rotation, and PRP was infused slowly; the final portion was deposited subcapsular during needle withdrawal.

Following bilateral injections, pelvic ultrasonography was performed to confirm vascular integrity and absence of free fluid. Patients were monitored for two hours post-procedure. ICSI was performed on retrieved oocytes, and all resulting embryos—including low-grade embryos—were cryopreserved (29).

Two weeks after PRP administration, serum AMH, FSH, and estradiol levels were reassessed, and AFC was evaluated via transvaginal ultrasound. If desired, patients underwent a second or third ICSI cycle. Endometrial preparation for frozen embryo transfer began on day 3 of the subsequent cycle with oral estradiol valerate (2 mg three times daily) until endometrial thickness exceeded 7 mm, after which one or two blastocysts were transferred depending on patient age (30).

Chemical pregnancy was defined as a positive serum β-hCG two weeks after embryo transfer, and clinical pregnancy was confirmed by visualization of fetal cardiac activity on transvaginal ultrasound five weeks after transfer (31).

Outcome Assessment

Primary outcomes included changes in AMH, FSH, estradiol, and AFC following PRP administration. Secondary outcomes included chemical and clinical pregnancy rates.

Statistical analysis

Statistical analyses were performed using SPSS software, version 23.0 (IBM Corp., Armonk, NY). The distribution of continuous variables was assessed using the Kolmogorov–Smirnov test. Depending on data normality, paired comparisons between pre- and post-intervention measurements were conducted using either the paired t-test for normally distributed variables or the Wilcoxon signed-rank test for non-normally distributed variables. A two-tailed P value <0.05 was considered statistically significant.

Results

This study included 15 infertile women diagnosed with diminished ovarian reserve, with a mean age of 36.00 ± 5.08 years. The demographic and baseline clinical characteristics of the participants are presented in Table 1 and Table 2. Following intraovarian PRP administration, a statistically significant increase in AMH levels was observed compared with baseline values ($P < 0.05$). Although FSH, estradiol, and AFC demonstrated numerical changes after treatment, these differences did not reach statistical significance.

Regarding reproductive outcomes, one participant achieved a chemical pregnancy that subsequently resulted in miscarriage, while another achieved a clinical pregnancy that progressed to a live birth. Comparative analysis of ovarian reserve parameters before and after PRP treatment is summarized in Table 3.

Table 1. Demographic characteristics of infertile women with reduced ovarian reserve

Characteristic		Value
Age (years)	< 35	8 (53.3%)
	≥ 35	7 (46.7%)
Body Mass Index (BMI, kg/m²)		28.93 ± 5.37
Type of Infertility	Primary	11 (73.3%)
	Secondary	4 (26.7%)
Duration of Infertility (years)	< 5	9 (60.0%)
	5 – 10	4 (26.7%)
	10 – 15	0 (0.0%)
	15 – 20	1 (6.7%)
	> 20	1 (6.7%)
Number of Previous IVF Cycles	1	7 (46.7%)
	2	5 (33.3%)
	3	3 (20.0%)

Data presented as mean ± standard deviation. All other data are presented as Number (Percentage).

Table 2. Clinical Features of Infertile Women with Reduced Ovarian Reserve

Clinical Feature	Value
Endometrial Thickness (mm)*	**7.42 ± 1.62**
Mean Days of Stimulation (on HCG Injection Day) *	8.93 ± 2.34

Number of Mature Oocytes *••	2.00 ± 1.30
Number of Retrieved Oocytes *	2.93 ± 2.89
Number of Total Injected Oocytes *	2.27 ± 2.49
Number of Total Embryos *	1.80 ± 2.30
**Transfer Type **	
Fresh	2 (13.3%)
Freeze	5 (33.3%)
No Transfer	8 (53.3%)
**Beta HCG **	
Positive	2 (13.3%)
Negative	13 (86.7%)

*: The data presented as mean ± standard deviation.
** :Data presented as number of patients (n) and percentage (%).
•• :Metaphase II (MII) refers to a mature oocyte that has completed its first meiotic division and is capable of being fertilized by a sperm cell.

Table 3: Comparison of Ovarian Parameters of Infertile Women Before and After PRP

Variable	Pre-treatment	Post-treatment	Test Statistic Value	P value
AMH (ng/mL)	0.54 ± 0.29	0.64 ± 0.55	t = -2.43 *	0.03
FSH (mIU/mL)	12.17 ± 11.37	13.95 ± 13.78	Z = -1.31	0.19
Estradiol (E2) (pg/mL)	71.33 ± 72.29	103.1 ± 171.79	Z = -0.94	0.35
AFC (count)	2.07 ± 1.22	3.60 ± 2.72	Z = -0.04	0.97

Data are presented as mean ± standard deviation.
*: $p < 0.05$ (significant)
AMH = Anti-Müllerian Hormone; FSH = Follicle-Stimulating Hormone; E2 = Estradiol; AFC = Antral Follicle Count.
Statistical Tests: A Paired Samples t-test was used for normally distributed data (AMH). The Wilcoxon Signed-Rank Test was used for non-normally distributed data (FSH, E2, AFC).
Note: The negative t-value for AMH indicates that the post-treatment mean was higher than the pre-treatment means. t value for the Paired t-test (used for AMH).
Z value for the Wilcoxon Signed-Rank Test (used for FSH, E2, AFC).

Discussion

In the present study, intraovarian PRP administration resulted in a significant increase in AMH levels, whereas changes in FSH, estradiol, and AFC did not reach statistical significance. These findings are consistent with the growing interest in PRP as a regenerative approach in reproductive medicine, as highlighted by recent clinical investigations (Sfakianoudis et al., 2022) (13); Bos Mikich et al., 2021) (33). Several studies have demonstrated

that PRP may enhance ovarian function through its rich concentration of growth factors and cytokines, which modulate tissue repair, angiogenesis, and follicular activation.

Previous research has reported variable effects of PRP on ovarian reserve markers. Hosseini et al. (2017) (34) suggested that PRP may stimulate follicular growth in women with diminished ovarian reserve. Similarly, Cakiroglu et al. (2020) (35) observed increases in AMH and AFC following PRP injection, while FSH levels remained unchanged, a pattern that aligns with the present findings. In contrast, Abdullah et al. (2019) (36) and Melo et al. (2020) (37) reported reductions in FSH and increases in AMH and AFC after PRP treatment. Differences between these studies and the current results may be attributed to variations in study design, duration of follow-up, socioeconomic characteristics of participants, and laboratory assay methods. For example, Melo et al. (2020) (37) conducted a two-year controlled study with a larger sample size and structured follow-up, which may explain the more pronounced treatment effects observed.

Evidence from pilot studies by Sfakianoudis et al. (2020) (38) in women with premature ovarian insufficiency, perimenopause, and poor ovarian response demonstrated significant improvements in hormonal profiles and ovarian reserve following PRP therapy. Similarly, Salih et al. (2020) (39) reported a notable increase in AMH levels after PRP administration, supporting the association between PRP and improved ovarian biomarkers. The increase in AMH observed in the present study is consistent with these findings, although the lack of significant change in other parameters may reflect the limited sample size and short follow-up period.

Spontaneous pregnancy rates in women with diminished ovarian reserve are generally low, ranging from 2.2% to 14.2% (40). In the present study, one chemical pregnancy ending in miscarriage and one clinical pregnancy resulting in a live birth were recorded. Previous reports have documented both successful and unsuccessful pregnancies following PRP treatment. Conversely, Hsu et al. (2020) (41) described a twin live birth in a 37-year-old woman with poor ovarian reserve following combined PRP and gonadotropin therapy, suggesting a potential synergistic effect between PRP and controlled ovarian stimulation. Since the present study evaluated only intraovarian PRP without adjunctive stimulation, differences in treatment protocols may explain the variation in pregnancy outcomes.

Cakiroglu et al. (2018) (42) reported 13 pregnancies and 9 live births among 311 women treated with PRP, while Melo et al. (2020) (37) found higher chemical and clinical pregnancy rates in PRP-treated women compared with controls. Variability in pregnancy outcomes across studies may be influenced by differences in sample size, patient characteristics, and PRP preparation techniques. It is also plausible that PRP may enhance luteal phase support or improve oocyte competence, contributing to improved reproductive outcomes.

This study has several limitations. The small sample size and pilot nature limit the generalizability of the findings. Additionally, some participants declined repeated PRP injections or follow-up testing due to concerns about treatment duration. Larger randomized controlled trials with longer follow-up periods are needed to better elucidate the therapeutic potential of PRP in women with diminished ovarian reserve.

Conclusion

Autologous PRP injection represents a minimally invasive therapeutic option that may offer potential benefits for women with diminished ovarian reserve, particularly those seeking to conceive using their own oocytes at advanced reproductive ages. The absence of surgical trauma and the biological compatibility of autologous PRP make it an appealing adjunct in fertility management.

Given the observed improvement in AMH levels in this study, further research is warranted to elucidate the underlying mechanisms through which PRP influences ovarian physiology. Future investigations should focus on characterizing the cytokines, growth factors, and molecular mediators involved in follicular activation and implantation following intraovarian or intrauterine PRP administration.

Additionally, well-designed clinical trials are needed to evaluate the potential role of PRP in ovulation-induction protocols and its possible contribution to luteal-phase support, which may enhance the likelihood of spontaneous pregnancy. Larger randomized studies with extended follow-up periods will be essential to determine the clinical efficacy, safety, and long-term reproductive outcomes associated with PRP therapy.

Abbreviations

AMH Anti Mullerian Hormone
AFC *Antral Follicle Count*
PRP Platelet Rich Plasma

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Generative AI
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Author' contributions
Conduct experiments and acquire data, M.Z, M.H, S.P; Writing – original draft, M.Z, M.H, F.S, S.P; Writing – review & editing, M.Z, M.H, F.S, S.P, M.A; Project administration, M.Z, M.H, M.A ; Conceptualization and Investigation, M.A, R.A.M, M.H.M, A.A ; Formal analysis, M.A, R.A.M, M.H.M, A.A; Study concept and Methodology, M.Z, M.H, S.P; Study supervision, M.Z, M.H, F.S, S.P. All authors reviewed the manuscript.

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Data availability
The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate
Present study was not blinding clinical trial, registered at Iranian Registry of Clinical Trials: IRCT20160815029374N7, This study was registered on 2019-06-20, conducted in Infertility Center, Imam Khomeini Hospital, Sari, Iran and approved by Ethical Committee at Mazandaran University of Medical Sciences: IR.MAZUMS.IMAMHOSPITAL.REC.1398.007. Also written informed consent was obtained from all patients. The study adhered to the ethical principles outlined in the Declaration of Helsinki (2013 revision). To ensure participant confidentiality and privacy, all collected data were anonymized and securely stored.

Consent for publication Not applicable.

Competing interests
The authors declare no competing interests.

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