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Intra-Ovarian Platelet-Rich Plasma in POSEIDON Group IV Poor Responders: A Pilot Study on ICSI Outcomes

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ABSTRACT

Background: Women classified as POSEIDON group IV poor responders face significant challenges with assisted reproductive technology (ART) because they produce few oocytes and have lower pregnancy rates. Researchers are investigating intra-ovarian injection of platelet-rich plasma (PRP) as a promising method to enhance their ovarian response to treatment. **Objective:** This study aimed to evaluate the effectiveness of intraovarian platelet-rich plasma (PRP) injection in improving laboratory outcomes and pregnancy rates in POSEIDON Group IV poor ovarian responders undergoing intracytoplasmic sperm injection (ICSI).

Patients and Methods: This pilot study involved 55 women categorized as POSEIDON group IV poor responders. The PRP group (n=25) received intra-ovarian PRP injections (3ml per ovary, prepared by buffy coat protocol) post-menstrually, one cycle prior to ICSI, in combination with gonadotropins. A control group (n=30) comprised women who declined PRP. Both groups subsequently underwent a GnRH antagonist ICSI protocol.

Results: While baseline demographics were similar, the PRP group exhibited a significantly longer duration of infertility (median 7.7 vs 4.0 years, p=0.026) and lower baseline E2 levels (median 26.8 vs 41.0 pg/ml, p=0.010). However, despite these differences and the use of PRP, there were no statistically significant variations between the PRP and control groups in key reproductive outcomes, including the number of retrieved oocytes (median 5 vs 6), fertilized oocytes (median 3 vs 2), transferred embryos (median 2 vs 1.5), hormonal levels on trigger day, endometrial thickness, or, crucially, the ongoing pregnancy rate (32.0% in PRP vs 16.7% in control, p=0.183). Other pregnancy outcomes and adverse events were also comparable between the groups.

Conclusion: It could be concluded that intra-ovarian PRP injection prior to ICSI does not demonstrate a statistically significant improvement in ovarian response parameters or pregnancy outcomes for POSEIDON group IV poor responders. Larger, randomized controlled trials are needed to further investigate its potential efficacy.

Keywords: Platelet-Rich Plasma; Poor Ovarian Responders; Ovarian Rejuvenation; Infertility.

INTRODUCTION

Poor ovarian response (POR) to controlled ovarian stimulation (COS) significantly complicates in vitro fertilization (IVF) for many women. Its reported incidence varies widely, ranging from 5.6% to 35.1% across different studies [(1-3)].

These patients typically yield a low number of oocytes, leading to fewer available embryos for transfer and consequently, diminished pregnancy rates. Defining POR has historically been complex due to patient heterogeneity and varied criteria. In 2016, the POSEIDON group established a classification system to better categorize fertility patients with a low prognosis. This system stratifies patients into four distinct groups based on their age, ovarian reserve markers (such as AMH and AFC), and their response to prior ovarian stimulation, aiming to reduce variability and enable more personalized and effective treatment strategies [(4)].

POSEIDON Group IV consists of women 35 or older with indicators of low ovarian reserve, such as an antral follicle count (AFC) under 5 or an anti-Müllerian hormone (AMH) level below 1.2 ng/mL, suggesting they will likely respond poorly to ovarian stimulation [(5)].

Managing these patients remains a debated issue in IVF, often with persistently low success rates despite various scientific and technological advances [(6,7)].

Platelet-Rich Plasma (PRP), derived from a patient's own blood, is gaining interest as a regenerative medicine treatment for Poor Ovarian Reserve (POR). PRP is concentrated with growth factors like Vascular Endothelial Growth Factor (VEGF), Transforming Growth Factor-beta (TGF-β), Platelet-Derived Growth Factor (PDGF), and Epidermal Growth Factor (EGF) [(8,9)]. Platelet-Rich Plasma (PRP) contains growth factors vital for tissue repair, blood vessel formation, and cell growth. Due to its regenerative properties, PRP has found applications in orthopedics, dentistry, and dermatology [(10)].

A new approach involves injecting PRP directly into the ovaries to potentially "rejuvenate" ovarian function in women with poor ovarian reserve (POR) or premature ovarian insufficiency (POI) [(11)]. This is based on the idea that PRP's concentrated growth factors could activate inactive follicles, improve the ovarian environment, and increase blood supply, ultimately enhancing the ovaries' response to stimulation [(12,13)].

Initial case series and small studies have reported promising outcomes after PRP treatment, including increased antral follicle counts, improved hormonal profiles, oocyte retrieval in previously non-responding patients, spontaneous pregnancies, and successful IVF outcomes [(14-16)]. Evidence supporting the use of intra-ovarian Platelet-Rich Plasma (PRP) is still largely preliminary. Further well-designed studies are necessary to standardize its preparation, establish the

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most effective dosage and timing, and thoroughly assess its efficacy and safety, especially in patient groups such as POSEIDON IV [17, 18]. Some research has hinted at the potential benefits of intra-ovarian PRP injections for poor responders, but more recent systematic reviews suggest these injections may not significantly improve live birth or clinical pregnancy rates. Therefore, this intervention remains experimental [19]. This pilot study was therefore designed to evaluate the effectiveness of intra-ovarian PRP injection on ovarian response parameters, laboratory variables, and pregnancy outcomes in women categorized as POSEIDON group IV poor responders undergoing ICSI.

PATIENTS AND METHODS

This pilot study included a total of 55 women complaining from infertility, attending at the Infertility Unit of Ain Shams University Maternity Hospital. This study was conducted between March 2021 to July 2022. Cases matching the inclusion and exclusion criteria among them 25 cases accepted to participate as the study group (PRP group) and the other 30 cases refused to the participate PRP group and they were enrolled as a control group.

Participants

This study focused on women aged 35 or older who were seeking infertility treatment and had specific indicators of low ovarian reserve: fewer than five antral follicles and an Anti-Müllerian Hormone (AMH) level under 1.2 ng/ml. Women fulfilling these criteria and willing to receive Platelet-Rich Plasma (PRP) treatment were included in the PRP group, while those who declined PRP served as the control group.

Exclusion criteria encompassed various factors that could impact ICSI success, such as patient refusal of PRP, first ICSI trial, severe male factor infertility, abnormal karyotyping, autoimmune disease history, other infertility etiologies (e.g., hydrosalpinx, significant endometrial pathology), and uncontrolled endocrinal disorders (e.g., diabetes mellitus, thyroid dysfunction, hyperprolactinemia). Ultimately, 55 women who met the inclusion criteria were enrolled, with 25 opting for the PRP group and 30 forming the control group.

PRP Preparation

Platelet-Rich Plasma (PRP) was prepared from 40 ml of autologous blood via a two-step centrifugation buffy coat method. The concentrated platelet pellet was then activated with 10% calcium gluconate.

PRP Intra-ovarian Injection (PRP Group)

In the postmenstrual period preceding the ICSI cycle, patients underwent an intraovarian PRP injection. Following intravenous administration of 1g ceftriaxone for pre-operative antibiotics and 100 mg propofol for sedation, a 17G x 330mm ovum pickup needle was guided into the ovarian stroma using transvaginal ultrasound. A total of 5ml of activated PRP was then combined with 1ml of 150 IU FSH/75 IU LH and slowly infused into the ovarian tissue, with 3ml administered into each ovary.

ICSI Protocol (Both Groups)

Patients undergoing controlled ovarian hyperstimulation (COH-ET) followed a flexible GnRH antagonist protocol. Ovarian stimulation began on day 2 with 450 IU/day of biosimilar FSH (Gonapure®), adjusted every 2-4 days based on ultrasound. A GnRH antagonist (Cetrotide® 0.25 mg) was added when a leading follicle was ≥ 12 mm, LH ≥ 10 IU/L, or E2 ≥ 150 pg/mL, and continued until hCG trigger. 10,000 IU of hCG (Choriomon®) was administered intramuscularly for final oocyte maturation once ≥ 3 follicles reached 17mm. Cycles with mono-follicular development or premature luteinization (elevated progesterone) were canceled or excluded. Hormonal profiles and endometrial thickness were monitored on day

Oocyte Retrieval, Fertilization, and Embryo Transfer

Oocytes were retrieved 36 hours post-HCG, followed by ICSI on metaphase II oocytes. Fertilization was confirmed by two pronuclei 16-19 hours later. Day 3 embryos or the highest-grade blastocysts (Gardner's grading) were selected for transfer.

Luteal Phase Support

Luteal phase support was provided with 400 mg of natural progesterone vaginal suppositories twice daily, starting from oocyte retrieval until the pregnancy test.

Outcome Measures

The study's primary outcome was to determine the ongoing pregnancy rate (pregnancies lasting over 20 weeks). Additionally, researchers tracked several secondary outcomes including hormonal levels (AMH, FSH, LH, E2, Prolactin), endometrial thickness, FSH ampoule usage, number of retrieved oocytes, fertilization rate, number of transferred embryos, and rates of chemical pregnancy, clinical pregnancy, and miscarriage. Procedure-related complications like infection and bleeding were also monitored.

Ethical Consideration:

This study was ethically approved by the Ain Shams University Faculty of Medicine's Ethical Committee (FMASU Ms 340/2021 and FWA 000017585). Written informed consent of all the participants was obtained. The study protocol conformed to the Helsinki Declaration, the ethical norm of the World Medical Association for human testing.

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics v23. Categorical data were analyzed with chi-squared or Fisher's exact tests, presented as counts and percentages. Mann-Whitney U-tests were used for numerical data, reported as median and interquartile range (IQR). Simple logistic regression calculated unadjusted odds ratios and pregnancy probability. A p-value less than 0.05 was considered statistically significant.

RESULTS

Patient Disposition and Baseline Characteristics

Fifty-five women meeting POSEIDON IV criteria were enrolled: 25 in the PRP group and 30 in the control group. The patient flow is detailed in Figure 1.

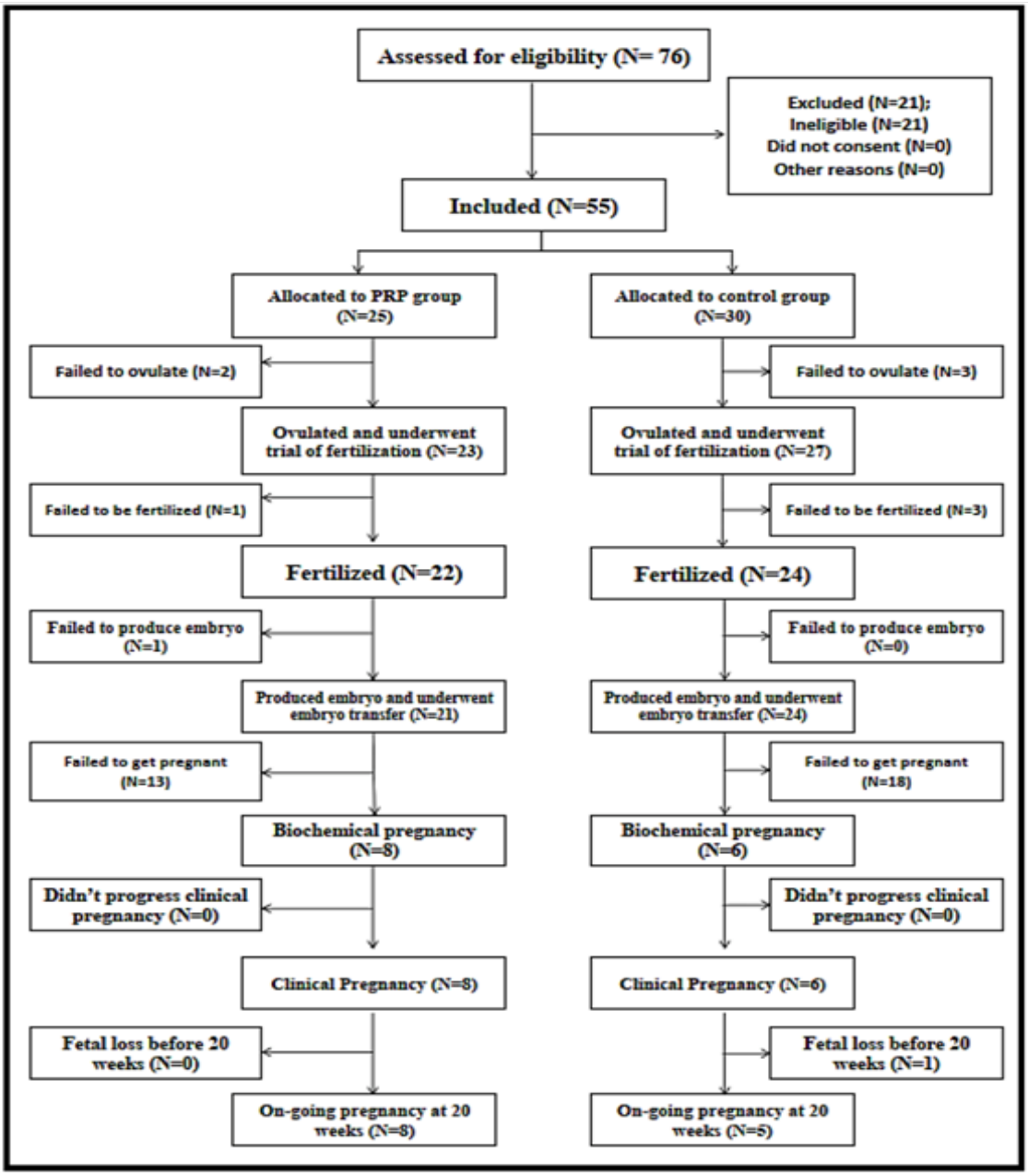

Figure (1): Flow chart showing patient enrollment, follow-up, and outcomes. (Description: Assessed for eligibility (N=76), Excluded (N=21), Included (N=55). PRP group (N=25): Failed to ovulate (N=2), Ovulated & trial of fertilization (N=23), Failed to be fertilized (N=1), Fertilized (N=22), Failed to produce embryo (N=1), Produced embryo & underwent transfer (N=21), Failed to get pregnant (N=13), Biochemical pregnancy (N=8), Didn't progress clinical pregnancy (N=0), Clinical Pregnancy (N=8), Fetal loss before 20 weeks (N=0), Ongoing pregnancy at 20 weeks (N=8). Control group (N=30): Failed to ovulate (N=3), Ovulated & trial of fertilization (N=27), Failed to be fertilized (N=3), Fertilized (N=24), Failed to produce embryo (N=0), Produced embryo & underwent transfer (N=24), Failed to get pregnant (N=18), Biochemical pregnancy (N=6), Didn't progress clinical pregnancy (N=0), Clinical Pregnancy (N=6), Fetal loss before 20 weeks (N=1), Ongoing pregnancy at 20 weeks (N=5)).

Table 1 shows that while age and BMI were comparable between the groups, the **PRP group** experienced a significantly longer duration of **infertility** (median 7.7 years) compared to the **control group** (median 4.0 years, p=0.026).

Table (1): Demographic characteristics of both study groups

Variable	PRP (N=25)	Control (N=30)	P-value†
Age (years), median (IQR)	38.0 (38.0 to 39.5)	39.0 (37.0 to 41.0)	0.558
BMI (kg/m²), median (IQR)	29.8 (28.0 to 31.3)	30.7 (26.2 to 34.3)	0.685
Duration of infertility (years), median (IQR)	7.7 (4.0 to 10.0)	4.0 (2.0 to 8.0)	0.026

†. Mann-Whitney test, IQR = interquartile range

Baseline Hormonal and Ultrasound Work-up

Table 2 shows baseline hormone levels and endometrial thickness. The PRP group had significantly lower serum E2 levels (median 26.8 pg/ml) compared to controls (41.0 pg/ml, p=0.010). Other baseline hormones (AMH, LH, FSH, prolactin) and endometrial thickness on the triggering day were not significantly different, though endometrial thickness trended higher in the PRP group.

Table (2): Results of hormonal and ultrasound work-up in both study groups

Variable	PRP (N=25)	Control (N=30)	P-value†
AMH (ng/ml), median (IQR)	0.7 (0.3 to 1.03)	0.6 (0.3 to 0.9)	0.487
E2 (pg/ml), median (IQR)	26.8 (16.1 to 41.9)	41.0 (31.7 to 54.5)	0.010
LH (mIU/ml), median (IQR)	5.3 (2.1 to 7.2)	5.8 (4.6 to 8.2)	0.196
FSH (mIU/ml), median (IQR)	10.2 (9.2 to 11.9)	8.6 (6.0 to 13.5)	0.060
Prolactin (µg/l), median (IQR)	12.5 (9.5 to 20.2)	12.1 (8.5 to 18.0)	0.636
Endometrial thickness on triggering day (mm), median (IQR)	10.4 (10.0 to 10.6)	9.5 (9.0 to 11.0)	0.055

†Mann-Whitney test, IQR interquartile range

Cycle Characteristics and Embryology Outcomes

PRP and control groups showed no significant differences in FSH ampoules, retrieved oocytes, fertilized oocytes, or transferred embryos (Table 3).

Table (3): Outcomes of induction of ovulation and fertilization in both study group

Variable	PRP (N=25)	Control (N=30)	P-value†
Number of FSH ampoules, median (IQR)	78 (54 to 90)	60.5 (48.0 to 72.0)	0.143
Number of retrieved oocytes, median (IQR)	5 (3 to 8)	6 (2 to 8)	0.677
Number of fertilized oocytes, median (IQR)	3 (1 to 5)	2 (1 to 4)	0.585
Number of transferred embryos, median (IQR)	2 (1 to 3)	1.5 (1 to 3)	0.136

† Mann-Whitney test, IQR = interquartile range

Hormonal assays on the triggering day showed no significant differences in E2 or progesterone levels between the two groups (Table 4).

Table (4) : Results of hormonal assay on triggering day in both study groups

Variable	PRP (N=25)	Control (N=30)	P-value†
E2 on triggering day (pg/ml), median (IQR)	710.0 (383.0 to 1243.8)	573.7 (246.0 to 774.5)	0.166
Progesterone on triggering day (ng/ml), median (IQR)	0.17 (0.06 to 0.79)	0.14 (0.06 to 0.18)	0.173

†Mann-Whitney test, IQR interquartile range.

Clinical Outcomes

While not statistically significant across all metrics, the PRP group exhibited a higher ongoing pregnancy rate at 20 weeks (32.0%) compared to the control group (16.7%), despite similar outcomes in ovulation, fertilization, embryo transfer, biochemical pregnancy, and clinical pregnancy rates.

Table (5) : Main outcome measures in both study groups

Variable	PRP (N=25)	Control (N=30)	P-value†
Ovulation, n (%)	23 (92.0%)	27 (90.0%)	1.000†
Fertilization, n (%)	22 (88.0%)	24 (80.0%)	0.487†
Fertilization rate (%), median (IQR)	60.0 (50.0 to 77.8%)	50.0 (23.1 to 76.9%)	0.219 ‡
Embryo transfer, n (%)	21 (84.0%)	24 (80.0%)	0.741个
Biochemical pregnancy, n (%)	8 (32.0%)	6 (20.0%)	0.3095
Clinical pregnancy, n (%)	8 (32.0%)	6 (20.0%)	0.3098
Ongoing pregnancy at 20 weeks, n (%)	8 (32.0%)	5 (16.7%)	0.183§

† Fisher's exact test, ‡ Mann-Whitney test, §. Pearson chi-squared test, IQR interquartile range, n number

Adverse Outcomes

The incidence of adverse outcomes was low in both groups (Table 6). There were no statistically significant differences in miscarriage or bleeding rates. No infections were recorded in either group.

Table (6): Incidence of adverse outcomes in both study group

Variable	PRP (N=25)	Control (N=30)	P-value†
Miscarriage, n (%)	0 (0.0%)	1 (3.3%)	>0.999
Bleeding, n (%)	0 (0.0%)	1 (3.3%)	>0.999
Infection, n (%)	0 (0.0%)	0 (0.0%)	NA

†. Fisher's exact test, n = number NA = test not applicable.

DISCUSSION

This pilot study evaluated intra-ovarian PRP injection in POSEIDON group IV poor responders before ICSI. Our findings showed no statistically significant improvements in most ovarian response parameters or pregnancy outcomes compared to a control group. A non-significant trend towards higher endometrial thickness on trigger day (10.4 mm vs. 9.5 mm) in the PRP group aligns somewhat with **Tülek *et al.*** [19]**.** While PRP contains growth factors, its direct intra-ovarian application may have limited systemic endometrial effects. Regarding ovarian response (FSH ampoules, retrieved oocytes, fertilized oocytes, transferred embryos), we found no significant differences, contrasting with **Farimani *et al.*** [11]**, Sfakianoudis *et al.*** [12]**, and Cakiroglu *et al.*** [14]**,** but, consistent with **Stojkovska *et al.*** [20]**.**

Animal studies e.g., **Dehghani *et al.*** [21] have shown increased follicle numbers, but this was not observed in our human cohort. The discrepancy might be due to differences in PRP preparation protocols, patient selection (though all these studies focus on poor responders), timing of PRP administration, or the inherent variability in pilot studies with smaller sample sizes. The POSEIDON IV criteria specifically select for patients with very low ovarian reserve, where the potential for follicular recruitment, even with growth factor stimulation, may be inherently limited.

No significant differences were observed in estradiol or progesterone levels on the trigger day. This is consistent with findings from **Farimani *et al.*** [11]**, Aflatoonian *et al.*** [18]**, and Stojkovska *et al.*** [20]**,** who also reported no significant changes in estradiol post-PRP.

Conversely, **Sfakianoudis *et al.*** [16] **and Tülek *et al.*** [19] did observe significant differences in E2 levels, suggesting that hormonal responses to PRP might be variable or dependent on specific patient or protocol factors not captured in our study.

The ongoing pregnancy rate was numerically higher in the PRP group (32.0%) compared to the control group (16.7%), although this difference was not statistically significant. Both biochemical and clinical pregnancy rates were similar across both groups, which aligns with findings from other studies [20, 22]**,** but, contradictory to others [11, 22] that reported higher rates with PRP. This highlights the ongoing debate about intra-ovarian PRP's clinical effectiveness. Our non-significant trend toward a higher pregnancy rate in the PRP group suggests a need for larger studies.

Adverse outcomes like miscarriage and bleeding were low and similar between groups, with no infections observed, aligning with the good safety profile of autologous PRP [23, 24]**.**

Limitations include a small sample size, potential selection bias as the control group refused PRP, and a longer duration of infertility in the PRP group, which could confound results. Our PRP protocol is also one of many.

Despite these, our pilot study adds to the literature on intra-ovarian PRP for POSEIDON IV poor responders, with detailed methodology for comparability. Future research should prioritize larger, multicenter randomized controlled trials (RCTs) to confirm efficacy, standardize PRP protocols, and investigate predictive biomarkers.

CONCLUSION

It could be concluded that intra-ovarian PRP injection, given one cycle before ICSI does significantly improve ovarian response, lab variables, or pregnancy outcomes in POSEIDON group IV poor responders compared to a

control group in this pilot study. While the PRP group had a small rise in ongoing pregnancy rates, larger randomized controlled trials are needed to confirm intra-ovarian PRP's benefit in these patients.

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