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The Effects of Combined Use of Ovarian Needle Puncture and Platelets Rich Plasma on Ovarian Total Antioxidants Capacity (TAC) Level and ICSI Outcome in PCOS Women

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Annotation: **Background:** Hyperandrogenism, ovulatory dysfunction, abnormal GnRH pulsation leading to irregular gonadotropin secretion all contribute to PCOS. Platelet Rich Plasma (PRP) are increasingly used for various disorders, including infertility, and is effective for ovarian rejuvenation via improving folliculogenesis.

Objectives: To estimate the effects of combined use of Ovarian Needle Puncture and PRP on ovarian total antioxidant capacity and ICSI cycle characteristics and outcome in PCOS Women

Patients and Methods: This study involved seventy women of infertile couples; patients were randomly allocated into two groups: **PRP Group (35 women):** Patients who underwent simultaneous use of Ultrasound-guided trans-vaginal ovarian needle puncture and PRP in the preceding cycle of ICSI cycle. **Non PRP Group (35 women):** Patients who didn't undergo simultaneous use of Ultrasound-guided trans-vaginal ovarian needle puncture and PRP

in the preceding cycle of ICSI cycle. CD7-CD8: Ultrasound-guided trans-vaginal ovarian needle puncture coincided with ovarian PRP was done under general light anesthesia for PRP group. Total antioxidant capacity was measured in the follicular fluid collected on the day of oocyte retrieval.

Results: There were no notable differences between PRP and Non-PRP groups in stimulation, oocyte, embryo characteristics, or ovarian total antioxidant capacity levels on ova pick up- day. Pregnancy rates were higher in the PRP group for both fresh (34.3% vs 20.0%, $P < 0.113$) and frozen ET (20.0% vs 14.3%, $P < 0.292$), though not statistically significant.

Conclusion: Although the combined use of ovarian needle puncture and platelet-rich plasma (PRP) did not produce significant effects on ovarian total antioxidant capacity (TAC) levels or on oocyte and embryo yield in women with PCOS, the cumulative pregnancy rate was higher following this intervention. These findings suggest the need for further large-scale studies to confirm the potential benefits of this approach.

Keywords: Polycystic ovary syndrome, Platelet Rich Plasma, Ultrasound-guided trans-vaginal ovarian needle puncture, Total antioxidant capacity.

Introduction

Polycystic ovary syndrome (PCOS) affects approximately 6–13% of women of reproductive age and has a hereditary component. Up to 70% of women with PCOS are undiagnosed worldwide. PCOS is the most common cause of anovulation and is a leading factor in infertility. The syndrome is associated with various long-term health conditions that impact both physical and emotional health[1]. The pathophysiological mechanisms by which PCOS negatively impact fertility are complex and not completely understood. Undoubtedly, hyperandrogenism, the consequent hyperestrogenemia, Insulin resistance IR, and compensatory hyperinsulinemia play an important role acting on both the ovary and the endometrium[2]. Oxidative stress OS plays a key role in the pathophysiology of a variety of gynecological disorders, including polycystic ovary syndrome (PCOS), endometriosis, unexplained infertility, and preeclampsia. The specific mechanism of Reactive Oxygen Species (ROS) production in PCOS is not fully understood, but several factors contribute to increased ROS production in individuals with PCOS[3]. In normal follicular fluid, oxidation occurs in a relatively balanced state; that is, there are not only ROS at the physiological level in follicular fluid but also a variety of antioxidant enzymes and non-enzymatic oxidants. As the main energy supply organ in follicular fluid, mitochondrial activity is

closely related to follicular quality. In the OS-activated state, the follicular fluid of PCOS patients produces excessive ROS, resulting in an imbalance of oxidation/antioxidation in the microenvironment and damaging the function of mitochondria in the follicular fluid[4]. Total antioxidant status (TAOS), which is defined as the ability of plasma/serum to inhibit free radical production, is a cumulative index of plasma antioxidant status and carbonyl content and should be considered when evaluating the effects of OS on the risk of developing cardiovascular diseases in women with PCOS[5]. Ovarian Total Antioxidant Capacity (TAC) is directly associated with reduced oocyte maturation and fertilization rates, poor embryo quality, and lower pregnancy rates. PCOS is associated with decreased antioxidant concentration and with increased OS, leading to disturbance in the cycle of ovarian follicular and luteal phases. Follicular fluid in women with PCOS demonstrated increased levels of ROS[6].

Platelets Rich Plasma (PRP) is a blood product that is prepared via a small amount of blood drawn from an individual and then centrifugation to obtain concentration about 5 times higher than that of the original whole blood[7]. Upon activation, platelets release alpha granules into the surrounding tissue, initiating a paracrine effect that contributes to various regulatory functions. These include the modulation of healing processes, immunomodulation, tissue regeneration, neoangiogenesis, homing, cellular apoptosis and viability, as well as analgesia, among other roles[8]. The mechanism of trans-vaginal ovarian needle punctures (UTND) is not fully understood, but it may resemble ovarian wedge resection or laparoscopic ovarian drilling (LOD). By puncturing the ovary and aspirating follicular fluid, UTND rapidly lowers intraovarian androgen levels in women with PCOS, whose ovarian tissues and fluids are typically rich in androgens[9].

Patients and methods

This is a prospective randomized (comparative) study was conducted in the High Institute for Infertility Diagnosis and Assisted Reproductive Technologies in AL Nahrain University, Baghdad – Iraq. The study started from the 1st of October 2023 till 1st of April 2025 involving seventy women of infertile couples attending the Infertility diagnosis clinic of the institute. The study was approved by the Local Medical Ethical Committee and written consent was obtained from each patient.

Inclusion Criteria: PCOS diagnosed according to Rotterdam's criteria and were eligible for Intracytoplasmic Sperm Injection (ICSI)

1. Hyperandrogism: Diagnosed either clinically by skin manifestations of androgen excess or hyperandrogenemia (high androgen test).
2. Ovulation dysfunction (i.e. Oligo/Anovulation).
3. Polycystic ovarian morphology by ultrasound examination.

PCOS infertile women could have other factors like tubal cause or prolonged duration of infertility rendering ICSI management were involved in this study.

PCOS woman of couple with a male factor indicating ICSI like severe Oligoasthozoospermia, Teratozoospermia, severe Oligoasthoteratozoospermia (OAT) were involved in the stud. Combined Factor Infertility: Included couples with infertile PCOS woman and husband with infertility cause, Age: 18- 40 years, BMI: 18-35, Platelet counts within accepted limits, (150000-400000) per microliter.

Exclusion Criteria: Medical or psychological disease that is contrary to pregnancy, Endometriosis, Untreated endocrine disorders, Untreated gynecological disease: pelvic infection, ovarian tumors, uterine factors of infertility, Patients with blood disorders and those taking antiplatelet or anticoagulant drugs and Patients who failed to gain Grade 1 embryos.

Patients were randomly allocated into two groups: **PRP Group (35 women):** Patients who underwent simultaneous use of Ultrasound-guided trans-vaginal ovarian needle puncture and

PRP in the preceding cycle of ICSI cycle. **Non PRP Group (35 women):** Patients who didn't undergo simultaneous use of Ultrasound-guided trans-vaginal ovarian needle puncture and PRP in the preceding cycle of ICSI cycle. All the females should have hormonal assay within the normal range measuring basal cycle day 2 FSH, LH, TSH, Prolactin, AMH, Androstenedione and Free Testosterone. CD7-CD8: Ultrasound-guided trans-vaginal ovarian needle puncture coincided with ovarian PRP was done under general light anesthesia for PRP group. The whole procedure of PRP preparation was carried out in an air-conditioned environment at 24 degrees Celsius.

PRP preparation broadly involves three main steps:

1. Sample collection PRP is derived from plasma, so the first step in preparing PRP is to generate plasma from the patient's blood. Approximately 20-30 mL of blood was collected from the patient by peripheral venipuncture to obtain 2-4 mL for PRP of both ovaries. Sodium citrate is the most recommended anticoagulant since it ensures better preservation of platelets[10, 11].

2. Centrifugation: Platelet-rich plasma (PRP) is derived from whole blood, which contains plasma (55%), red blood cells (41%) platelets and white blood cells (4%), by centrifugation and separation of its different components. The centrifugation and separation process leads to the removal of red blood cells and the production of plasma with 5–10 times higher concentrations of growth factors[12].

The rotor's size and radius (R) vary with different centrifuge machines. Therefore, relative centrifugal force (g) values are converted to rotation per minute (rpm). The conversion factor from 'g' to rpm is as follows: $g = (1.118 \times 10^{-5}) R (rpm)^2$ [13]. After centrifugation (1800*g* for 6 minutes). The upper layer corresponding relatively to platelet-poor plasma was aspirated and discarded. The layer of buffy coat was accessed layer was aspirated and placed in a separate sterile conical tube for another round of centrifugation, and the lower level corresponding to red blood cells was discarded. The process was repeated a second time; no activators were added to the final 4 mL of PRP were aspired to a sterile syringe.

3. Storage: Maintaining the quality of the PRP prior to its clinical application. Ideally, PRP should be used within maximum 8 hours of centrifugation, as this maintains the leukocyte concentration and pH of solution[19]. For our patients PRP was injected within one hour.

Under intravenous sedation or general anesthesia by a licensed anesthesiologist and using ultrasound guided transvaginal injection, intraovarian injection of approximately 2 mL of PRP per ovary was performed. The injection was performed in multifocal spots and the diffusion of the PRP in the subcortical layers was achieved by applying 3 to 4 punctures per ovary transvaginally using a 22-gauge needle and guide[18]. Post operatively, the patient tolerated the procedure well and discharged on antibiotics and minor analgesic tablets. All women instructed to attend the Infertility Clinic in the institute to start ICSI cycle in cycle day two.

Controlled Ovarian Hyperstimulation Protocols: As all women are with PCOS, the flexible antagonist protocol type was employed for ICSI[14]. Stimulation started on CD2 by administration of daily subcutaneous injections of rFSH (Gonal F, Merk Serono) in a dose ranged between 75 IU- 225IU per day in a fixed time. When a minimum of three follicles reached the size equal to or more than 17–18 mm, ovulation and final oocytes maturation was triggered by either one of two protocols keeping the aim of OHSS avoidance: **A. The Dual Trigger**: Applied by administration of recombinant hCG (Ovitrelle®, 250 IU Merk serono, Switzerland) and 0.2mg Triptorelin (Decapeptyl; Ferring Pharmaceuticals) administered subcutaneously thirty-five hours before ova pick-up time. **B. GnRH Agonist Trigger**: The primary clinical benefit of a GnRH-a trigger is its ability to induce rapid, reversible luteolysis, reducing the risk of OHSS. Patients received a single 0.2 mg bolus of GnRH-a (triptorelin; decapeptyl, Ferring) for final oocyte maturation. Freezing all embryos after GnRH-a trigger and transferring in a next cycle that is called segmentation of IVF cycle. This procedure had been used with in women who were

exposed to risk of OHSS[15].

The specialist in ARTs recovered the oocytes 34–36 hours post-triggering, just prior to follicular rupture. Patients were admitted to the operating theatre in a fasting state; Oocytes were retrieved from individuals under transvaginal ultrasound guidance The entire procedure typically lasted between 20-30 minutes. Subsequently, patients received analgesics, antibiotics, and luteal phase support in the form of proge-sterone. Women who received Dual trigger underwent Fresh Embryo transfer and the same method for embryo transfer was applied in the case of Frozen embryo transfer for women with GnRH agonist trigger in both the PRP and groups. The selected number of embryos for transfer was determined according to the embryonic quality, the age of the patient, rank of the attempt, and the clinical history. For the first attempt in women under 35 years, only 2 embryos were transferred. For women over 35 years we transferred 3 embryos. A pregnancy test was done approximately 2 weeks after embryo transfer to check for successful implantation.

Sampling: Follicular fluid samples were taken to assess Ovarian Total Antioxidants Capacity (OTAC). The follicular fluid seemed to be macroscopically clear without any blood contamination and was clarified by centrifugation at1800*g* for 10 minutes successively to eliminate cells and cell debris, respectively. The supernatant fluids were then stored at −70°C until further analysis. Human Total antioxidant capacity (T-AOC) ELISA Kit. was used. This kit uses enzyme-linked immune sorbent assay (ELISA) based on the Biotin double antibody sandwich technology to assay the Human Total antioxidant capacity (T-AOC).

Results

1. Demographic Characteristics

Seventy patients were enrolled in this study, with thirty-five patients assigned to the Platelet-Rich Plasma (PRP) group and thirty-five patients assigned to the non-PRP (control) group. The demographic characteristics of both groups are systematically presented in Table 1.

Table 1: Comparison of Demographic Characteristics between PRP group and non-PRP group

Characteristic	PRP group	non-PRP group	*P*
	35 cases	35 cases	
Age (years			
Mean ±StDe.	28.71 ±5.36	29.43 ±5.54	0.585 N
Min.-Max.	18 -39	19 -39	
BMI (kg/m^2)			
Mean ±StDe.	27.72 ±3.73	28.31 ±3.16	0.480 N
Min.-Max.	21 -33.7	21 -35	
Duration of Infertility (years)			
Mean ±StDe.	6.49 ±4.01	7.74 ±3.76	0.181 N
Min.-Max.	1 -20	2 -18	
Type of Infertility			
Primary	29 (82.9 %)	26 (74.3 %)	0.382 N
Secondary	6 (17.1 %)	9 (25.7 %)	
Cause of Infertility			
Male Factor	17 (48.6 %)	13 (37.1 %)	0.488 N
Female Factor	4 (11.4 %)	3 (8.6 %)	
Dual Factor	14 (40.0 %)	19 (54.3 %)	

StDe.: standard deviation; **BMI**: body mass index; **N**: not significant

No statistically significant differences were observed in mean age, mean body mass index, mean

infertility duration, or in the distribution of patients by infertility type or cause ($p > 0.05$).

2. Serum Hormones Levels on Cycle Day 2

Comparison of mean serum hormonal levels on cycle day 2 between PRP group and non-PRP group is shown in Table 2.

Table 2: Comparison of Mean Serum Hormonal Levels on Cycle Day 2 between PRP group and non-PRP group

Characteristic	PRP group	non-PRP group	*P*
	35 cases	35 cases	
CD2 FSH (mIU/ ml)			
Mean ±StDe.	5.65 ±1.08	5.49 ±1.60	0.633 N
Min.-Max.	2.8 -7.3	2.88 -11.2	
CD2 LH (mIU/ ml)			
Mean ±StDe.	5.11 ±1.92	5.88 ±2.11	0.115 N
Min.-Max.	1.6 -8.7	1.9 -12.9	
TSH (pg/ml)			
Mean ±StDe.	1.89 ±0.58	1.98 ±0.60	0.536 N
Min.-Max.	0.56 -3	0.63 -2.7	
Prolactin (mIU/ ml)			
Mean ±StDe.	19.64 ±5.02	17.46 ±6.65	0.125 N
Min.-Max.	7 -29	3.2 -32	
E2 (pg/ml)			
Mean ±StDe.	30.50 ±11.09	33.89 ±10.21	0.187 N
Min.-Max.	12 -51.2	10 -52	
AMH (ng/ml)			
Mean ±StDe.	4.53 ±1.69	4.70 ±1.41	0.641 N
Min.-Max.	1.6 -9.2	2.87 -9.78	
Free Testosterone (pg/ml)			
Mean ±StDe.	1.35 ±0.91	1.61 ±1.50	0.379 N
Min.-Max.	0.12 -4.15	0.09 -7.65	
Androstenedione (ng/ml)			
Mean ±StDe.	1.88 ±0.98	2.30 ±1.04	0.088 N
Min.-Max.	0.32 -4.50	0.77 -5.9	

StDe.: standard deviation; **N**: not significant; **CD2:** cycle day **2; FSH:** Follicle Stimulating Hormone**; LH:** Luteinizing Hormone**; TSH:** Thyroid Stimulating Hormone**; E2:** Estradiol**; AMH:** Anti-mullerian Hormone.

Table 2 showed lack of significant variations in mean levels of FSH, LH, TSH, prolactin, estradiol and AMH ($p > 0.05$). There was no statistically significant difference in mean free testosterone levels between the PRP group and the non-PRP group prior to PRP administration, as measured on cycle day 2 ($p = 0.379$). Similarly, the mean androstenedione values did not differ significantly between the non-PRP and PRP groups ($p = 0.088$).

3. Comparison of ICSI Cycle Stimulation Characteristics

Stimulation characteristics are shown in Table 3 and comparison revealed no significant differences in mean dose of FSH, mean number of GnRH Antagonist Ampoules, mean day of trigger, mean E2 at trigger day, and trigger type ($p > 0.05$).

Table 3. Comparison of ICSI Cycle Stimulation Characteristics between PRP group and non- PRP group

Characteristic	PRP group 35 cases	non-PRP group 35 cases	*P*
Dose of FSH			
Mean ±StDe.	1726.40 ±449.55	1727.10 ±488.86	0.995 N
Min.-Max.	1150 -3000	1050 -3100	
Number of GnRH Antagonist Ampoules			
Mean ±StDe.	3.46 ±0.78	3.26 ±0.66	0.250 N
Min.-Max.	2 -5	2 -5	
Trigger day			
Mean ±StDe.	10.63 ±0.91	10.57 ±0.65	0.764 N
Min.-Max.	9 -13	9 -12	
E_2 on Trigger Day (pg/ml)			
Mean ±StDe.	2443.00 ±860.16	2429.20 ±949.92	0.949 N
Min.-Max.	1255 -4360	1008 -6680	
Trigger type			
Dual trigger	20 (57.1 %)	17 (48.6 %)	0.473 N
Agonist Trigger	15 (42.9 %)	18 (51.4 %)	

N: not significant

There was no significant difference in proportion of patients with Ovarian Hyperstimulation Syndrome (OHSS) on day of ova pick up (*p* 0.172), as shown in Figure 1.

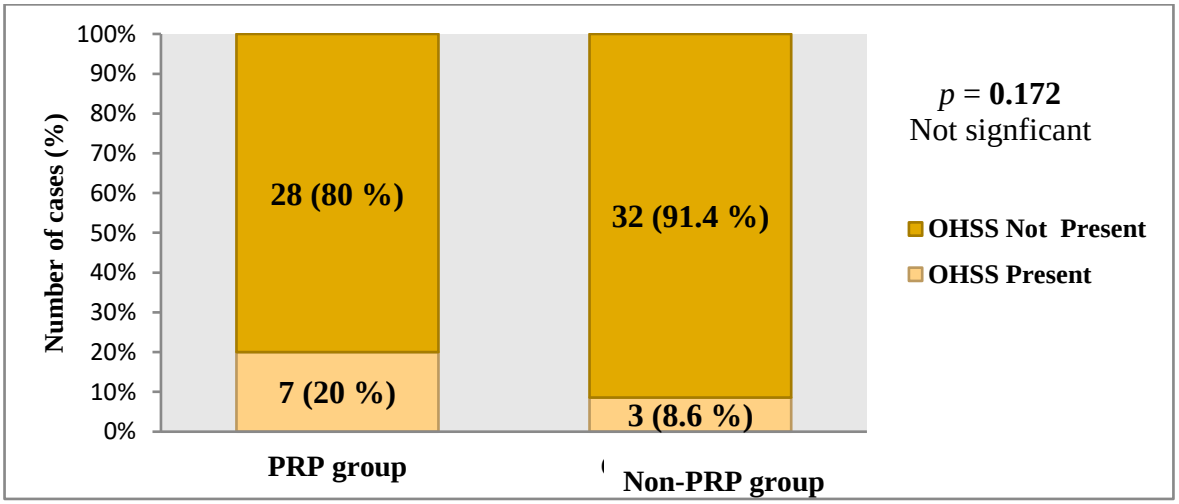

Figure 1: Comparison of Ovarian Hyperstimulation Syndrome (OHSS) Incidence Rates

4. Comparison of Oocytes Characteristics

Table 4 presents the characteristics of oocytes. The results indicated that there were no statistically significant differences in the mean number of follicles, mean number of aspirated oocytes, mean number of mature oocytes at metaphase II stage (MII), mean number of immature oocytes metaphase I stage (MI), mean number of germinal vesicle oocytes, mean number of abnormal oocytes, follicle-to-oocyte ratio, and maturation index ($p > 0.05$).

Table 4: Comparison of Oocytes Characteristics between PRP group and non- PRP group

Characteristic	PRP group 35 cases	non-PRP group 35 cases	*P*
Number of Follicles in Ova pick up			
Mean ±StDe.	23.89 ±8.36	24.97 ± 9.97	0.623 N
Min.-Max.	9 -44	11 -58	
Number of Oocytes on Ova pick up			
Mean ±StDe.	17.86 ±7.39	17.43 ±8.01	0.817 N
Min.-Max.	4 -34	8 -51	
Mature (MII) Oocytes			
Mean ±StDe.	13.09 ±6.07	12.86 ±6.98	0.884 N
Min.-Max.	1 -25	3 -40	
Immature metaphase I (MI) oocytes			
Mean ±StDe.	0.94 ±1.16	0.94 ±1.11	1.000 N
Min.-Max.	0 -4	0 -4	
Germinal vesicle (GV) oocyte			
Mean ±StDe.	2.26 ±1.95	2.23 ±2.12	0.953 N
Min.-Max.	0 -7	0 -8	
Abnormal Oocytes			
Mean ±StDe.	1.51 ±2.51	1.06 ±1.43	0.353 N
Min.-Max.	0 -12	0 -5	
Follicle to oocyte ratio			
Mean ±StDe.	74.55 ±16.13	69.86 ±10.06	0.149 N
Min.-Max.	17.4 -93.3	43.8 -87.9	
Maturation index			
Mean ±StDe.	73.28 ±16.81	72.79 ±18.49	0.909 N
Min.-Max.	7.1 -100	18.8 -100	

N: not significant

5. Comparison of Embryos Characteristics

A comparison between the non-PRP and PRP groups showed in Table 5 and revealed no significant differences in 2PN oocyte count, fertilization rate, mean numbers of grade 1–4 embryos, frozen embryos, transferred embryos, or type of embryo transfer ($p > 0.05$).

Table 5: Comparison of Embryos Characteristics between PRP group and non-PRP group

Characteristic	PRP group 35 cases	non-PRP group 35 cases	*P*
Number of 2PN			
Mean ±StDe.	9.77 ±5.32	10.77 ±6.25	0.474 N
Min.-Max.	1 -19	2 -35	
Fertilization rate			
Mean ±StDe.	75.31 ±19.00	81.25 ±17.48	0.178 N
Min.-Max.	15.8 -100	28.6 -100	
Number of Grade 1 Embryos			
Mean ±StDe.	6.29 ±4.19	6.89 ±4.10	0.547 N
Min.-Max.	1 -15	2 -23	
Number of Grsde 2 Embryos			
Mean ±StDe.	1.44 ±1.60	1.86 ±1.88	0.327 N
Min.-Max.	0 -8	0 -8	
Number of Grade 3 Embryos			

Mean ±StDe.	0.60 ±0.95	0.97 ±1.32	0.180 N
Min.-Max.	0 -4	0 -6	
Number of Grade 4 Embryos			
Mean ±StDe.	1.57 ±1.94	1.09 ±1.54	0.251 N
Min.-Max.	0 -7	0 -6	
Number of Frozen Embryos			
Mean ±StDe.	7.38 ±3.62	5.39 ±5.96	0.154 N
Min.-Max.	1 -19	0 -31	
Number of Transferred Embryos			
Mean ±StDe.	2.26 ±0.51	2.43 ±0.50	0.159 N
Min.-Max.	1 -3	1 -3	
Type of Embryo Transfer			
Fresh Embryo Transfer	18 (51.4 %)	17 (48.6 %)	0.811 N
Frozen Embryo Transfer	17 (48.6 %)	18 (51.4 %)	

N: not significant

6. Comparison of Follicular Fluid Total Antioxidants Capacity (TAC) levels between PRP group and non-PRP group

A comparison was conducted to evaluate the follicular fluid total antioxidant capacity (TAC) levels between the PRP group and the non-PRP group. As shown in Table 6, the analysis revealed no statistically significant difference in TAC levels between the two groups (p = 0.388).

Table 6: Comparison of Follicular Fluid TAC Levels between PRP group and non-PRP group

Characteristic	PRP group	non-PRP group	*P*
	35 cases	35 cases	
Follicular fluid TAC			
Median (IQR)	6.1 (3.92)	5.6 (3.1)	0.388 N
Min.-Max.	1.19 -113.2	2.1 -49	

IQR: inter-quartile range; **N**: not significant

7. Comparison of Pregnancy Rate between PRP group and non-PRP group

When examining pregnancy rates following fresh embryo transfer, the PRP group demonstrated a higher rate of pregnancy after fresh embryo transfer (12 cases, 34.3%) than the non-PRP group (7 cases, 20.0%). Similarly, the pregnancy rate after frozen embryo transfer was also higher in the PRP group (7 cases, 20.0%) compared to the non-PRP group (5 cases, 14.3%).

Table 7: Comparison of Pregnancy Rate between PRP group and non-PRP group

Characteristic	PRP group	non-PRP group	*P*
	35 cases	35 cases	
Not Pregnant	16 (45.7 %)	23 (65.7 %)	Reference
Pregnant after fresh ET	12 (34.3 %)	7 (20.0 %)	0.113 N
Pregnant after frozen ET	7 (20.0 %)	5 (14.3 %)	0.292 N

Discussion

To date there are few studies investigating the role of PRP in PCOS women and relatively more studies explained cold Ultrasound-guided Transvaginal Ovarian Needle Puncture. This study investigated the effect of applying a relatively less invasive method to the PCOS women by applying limited number of needle punctures and injecting PRP into the ovary guided by Transvaginal Ultrasound.

1. Demographic Characteristics: In this study, participants were randomly assigned to groups, resulting in no statistically significant differences in mean age, mean of body mass index, mean duration of infertility, or in the distribution of patients by type and cause of infertility. Such equivalence between groups is essential in interventional case-control studies to minimize potential bias in outcome assessments.

2. Serum Hormones Levels on Cycle Day 2: In this study, comparison of mean serum hormonal levels, FSH, LH, Prolactin, E_2 and AMH, Free Testosterone and Androstenedione baseline levels in cycle day two between non-PRP group and PRP group revealed lack of significant variations. These findings is most likely due to random distribution of patients on PRP and Non-PRP groups.

3. Comparison of ICSI Cycle Stimulation Characteristics: In the present study, no significant differences were observed between the PRP and non-PRP groups regarding the mean dose of FSH, mean number of GnRH antagonist ampoules, mean day of trigger, mean E2 at trigger day, or trigger type. Additionally, there was no significant difference in the proportion of patients experiencing Ovarian Hyperstimulation Syndrome (OHSS). This finding is attributed to the successful random allocation of participants into the PRP and non-PRP groups and denied the questioned role of the study intervention in increasing risk of OHSS.

4. Comparison of Oocytes Characteristics: The present study demonstrated no significant differences between the PRP and non-PRP groups in oocyte characteristics, including the mean number of follicles, mean number of oocytes, mean number of mature oocytes (MII), mean number of immature oocytes (MI), mean number of germinal vesicle oocytes, mean number of abnormal oocytes, follicle-to-oocyte ratio, and maturation index. In 2024, Shrivastava et al., demonstrated that PRP treatment resulted in an increased follicle count, improved oocyte quality, and a successful pregnancy in IVF cycles [16]. In PCOS, increased collagen deposition results in a stiffened ovarian cortex, creating a mechanically restrictive environment in vivo that can adversely affect intracellular signaling pathways. The mechanical tension produced by the rigid stromal architecture may hinder normal folliculogenesis, ultimately affecting the developmental competence of ovarian follicles. From a clinical perspective, procedures such as ovarian drilling (including wedge resection or diathermy/laser drilling) in polycystic ovary syndrome (PCOS) ovaries—disruptive interventions by nature—may contribute to the interruption of Hippo signaling through the facilitation of actin polymerization, thereby promoting further follicular development[17, 18, 19].

5. Comparison of Embryos Characteristics: Analysis revealed no statistically significant differences in the number of 2PN oocytes, fertilization rates, mean counts of grade 1–4 embryos, numbers of frozen and transferred embryos, or the type of embryo transfer procedure. It should be noted that this study relied exclusively on morphological assessments of embryos, without incorporating genetic or molecular analyses. As a result, subtle genetic or molecular changes that may influence oocyte and embryo quality could not be detected using conventional morphological evaluation alone so as, the possibility of underlying genetic or molecular improvements associated with the intervention cannot be entirely excluded. In line with such suggestions, according to a recent study, transvaginal ovarian drilling (TVOD) plus intraovarian PRP (ovarian rejuvenation) significantly increased the percentage and possibly the yield of euploid blastocysts. Additionally, TVOD+PRP significantly reduced the number of aneuploid embryos compared to PRP alone [20].

6. Comparison of Follicular Fluid Total Antioxidants Capacity (TAC) levels between PRP group and non-PRP group: In the present study, follicular fluid total antioxidant capacity (TAC) levels were similar between the non-PRP and PRP groups, indicating no significant difference attributable to PRP treatment. However, this lack of significant variation in TAC should not be construed as evidence against the potential impact of PRP on oxidative stress and ovarian antioxidant capacity. The scope of current investigation was limited to TAC, while other

oxidative stress markers were utilized by other studies[21]. A 2019 study demonstrated that targeted platelet-rich plasma (PRP) administration significantly increased ovarian total antioxidant capacity (TAC) and reduced the lipid peroxidation ratio. Conversely, reductions in tissue levels of antioxidant enzymes—such as superoxide dismutase (SOD), glutathione peroxidase (GSH-px), and catalase—have been associated with the initiation and progression of oxidative stress in ovarian tissue. The research measured tissue concentrations of SOD and GSH-px to assess these changes. Findings indicated that PRP supplementation substantially restored GSH-px and SOD levels diminished by polycystic ovary syndrome (PCOS). Based on biochemical data, the authors concluded that PRP can upregulate ovarian GSH-px and SOD levels, markedly reduce mRNA damage, and lower ovarian malondialdehyde (MDA) content[22].

One proposed mechanism for PRP's effectiveness is its ability to reduce oxidative stress in follicular fluid. In vitro, studies have shown that PRP significantly impacts granulosa cells, promoting high proliferation rates and upregulating genes essential for reproduction[23, 24]. PRP has been shown to reduce abnormal ROS accumulation by improving mitochondrial function thereby protecting oocytes and granulosa cells from oxidative damage. In vitro studies have confirmed that PRP helps maintain mitochondrial activity in mature oocytes[25]. In vitro studies have shown that PRP significantly impacts granulosa cells, promoting high proliferation rates and upregulating genes essential for reproduction[26]. Oxidative imbalance worsens numerous diseases, and recent studies suggest that PRP administration enhances antioxidant capacity, offering both protective and therapeutic benefits[27, 28,29,30].

7. Comparison of Pregnancy Rate between PRP group and non-PRP group: In this study, pregnancy rates following fresh and frozen embryo transfer, the PRP group demonstrated a higher rate of pregnancy, the pregnancy rate was 54.3% in the PRP group compared to 34.3% in the non-PRP group, which is clinically significant. While the observed difference did not reach statistical significance, it may still hold relevance in the context of clinical outcomes for infertility management. The objective of applying minimally invasive mechanical stimulation via needle puncture, combined with the potential benefits of PRP, warrants further consideration regarding its impact on pregnancy rates.

Conclusion

Although the combined use of ovarian needle puncture and platelet-rich plasma (PRP) did not produce significant effects on ovarian total antioxidant capacity (TAC) levels or on oocyte and embryo yield in women with PCOS, the pregnancy rate was higher following this intervention. These findings suggest the need for further large-scale studies to confirm the potential benefits of this approach.

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