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Effects of platelet-rich plasma and granulocyte colony-stimulating factor intra ovarian injection on enhancing ovarian reserve parameters

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SUMMARY

Background: Poor ovarian reserve significantly reduces reproductive potential by lowering ovarian follicle quantity and quality. Many recent therapeutic strategies have shown potential benefits in improving ovarian function.

Objective: To evaluate the effects of platelet-rich plasma, growth-stimulating factors, and their combination injection on ovarian function parameters in females with diminished ovarian reserve.

Patients and method: A prospective analytical (clinical trial) study was conducted on infertile females referred to the Infertility Department of Higher Institute for infertility Diagnosis and Assisted Reproductive Technologies, Baghdad, Iraq between July 2023 and December 2024. Sixty females aged 25–45 years who were diagnosed to have poor ovarian reserve were randomly divided into three equal groups depending on injected substance on day 6-7 of the menstrual cycle into platelet-rich plasma, Granulocyte Colony-Stimulating Factor, and combined therapy (PRP + GSF) groups. Primary outcome included ovarian artery Resistance Index, Anti-Müllerian Hormone, antral follicle count, and follicle size. Secondary outcome involved an assessment of parameters 1–3 months post-intervention.

Results: Platelet-rich plasma alone significantly reduced ovarian Resistance Index (p=0.034). Granulocyte Colony-Stimulating Factor significantly increased Antral Follicle Count (p=0.027), indicating improved follicular recruitment. The combined treatment (PRP + GSF) significantly decreased Resistance Index (p<0.001), increased Antral Follicle Count (p=0.002), and enhanced follicle size (p=0.002). AMH changes varied, suggesting individualized responses.

Conclusion: Platelet-rich plasma, Granulocyte Colony-Stimulating Factor, and their combination significantly improved ovarian function.

Keywords: PRP; GSF; Ovarian reserve; Ovarian resistance index

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INTRODUCTION

The quantity of oocytes that remain in the ovary is known as the ovarian reserve. Female newborns have between 500,000 and 1 million oocytes at birth; ovulation and follicular atresia cause the number of oocytes to gradually decrease over time, and menopause follows. Although ovarian reserve and age have an inverse relationship, women of the same chronological age differ significantly in their ovarian reserve [1]. The main factor limiting success in both spontaneous conception and Assisted Reproductive Technology (ART) is a gradual decrease in the number and quality of oocyte reserves linked to ovarian aging [2,3].

Poor Ovarian Reserve (POR) indicates a reduction in the quantity of ovarian follicular pool in women of reproductive age group and is an important cause of infertility in many couples [4,5]. Women in their mid to late thirties are frequently observed to have diminished ovarian reserve, but younger women may also be affected. It is thought that the follicular pool experiences a rapid decline when it falls below a threshold value of 25,000 between the ages of 37 and 38. There is therefore still a very short window of time for using one's own eggs for conception [6,7].

Identifying POR is crucial, as it lowers pregnancy rates and increases pregnancy loss. While a shortened menstrual cycle may indicate POR, it is not a reliable diagnostic criterion [8,9]. Various ovarian reserve tests have been in use to assess ovarian reserve and predict response to ovarian stimulation [10,11]. Antral Follicle Count (AFC) and Anti-Müllerian Hormone (AMH) are the most sensitive ovarian reserve markers [12], ideal for personalized ovarian stimulation. They accurately predict ovarian response and are interchangeable for clinical use [13].

Most definitions of Poor Ovarian Response (POR) in IVF are based on low peak estradiol (300–500 pg/ml), ≤ 5 follicles, or ≤ 5 eggs. Some also consider age ≥ 40, abnormal Ovarian Reserve Tests (ORT), or prior poor response [14-16]. POR is typically diagnosed retrospectively after at least one IVF cycle. By 1999, 35 definitions had been documented [17]. The Bologna criteria were established in 2011 by the ESHRE working group on POR definition to standardize the diagnosis of poor ovarian response. To diagnose POR, at least two of the following three conditions must be met: (1) maternal age ≥ 40 years, (2) previous poor ovarian response (≤ 3 oocytes retrieved in standard stimulation), or (3) abnormal ovarian reserve test (AMH: 0.5–1.1 ng/ml or AFC: 5–7 follicles) [18].

Recent developments in the field of reproductive medicine have looked at Platelet-Rich Plasma (PRP) and growth factor-stimulating factors as therapeutic agents to treat poor ovarian reserve. PRP injection is the process of injecting autologous growth factor-enriched plasma into the ovaries to restore follicular function and improve oocyte quality. Literature indicates that PRP injection within the ovary could improve the function of the ovaries and fertility factors in women with poor ovarian reserve [19]. Platelet-rich plasma enhances poor ovarian reserve by delivering growth factors (VEGF, IGF-1, PDGF, TGF-β, EGF) to ovarian tissue, promoting angiogenesis, granulosa cell proliferation, and reducing inflammation. It activates dormant follicles *via* PI3K-Akt signaling and may stimulate ovarian stem cells, improving follicular growth, tissue regeneration, and ovarian function [14]. Additionally, growth-stimulating factors such as granulocyte colony-stimulating factor (G-CSF) have been investigated for their role in enhancing endometrial receptivity and ovarian response, with some studies reporting improved implantation rates in POR patients undergoing IVF [11]. G-CSF enhances ovarian function by activating G-CSFR in granulosa cells, promoting follicular growth, maturation [20], and survival. It boosts blood supply (*via* VEGF), reduces apoptosis (PI3K-Akt, MAPK), and lowers inflammation, improving follicular recruitment, oocyte quality, and ovarian responsiveness [21].

Platelet-Rich Plasma (PRP) is enriched with a high concentration of platelets, which release growth factors like PDGF, VEGF, and TGF-β. Growth factors are essential for normal cellular functions, such as tissue development, maintenance, and healing [22]. Fibroblast Growth Factors (FGFs) stimulate angiogenesis and neurogenesis [23], while Vascular Endothelial Growth Factor (VEGF) promotes the formation of new blood vessels in response to hypoxia [24]. Epidermal Growth Factor (EGF) stimulates wound healing and skin regeneration, and Platelet-Derived Growth Factor (PDGF) encourage collagen production in tissue repair [25]. However, when growth factor signaling becomes dysregulated, it can lead to pathological lead to the development of cancer by promoting uncontrolled cell proliferation. For example, Epidermal Growth Factor Receptor (EGFR) mutations are often associated with several cancers, including lung and colorectal cancer [26].

The primary aim of this study is to evaluate the effects of Platelet-Rich Plasma (PRP), Granulocyte Colony-Stimulating Factor (GSF), and their combined administration (PRP + GSF) on ovarian function in women diagnosed with diminished ovarian reserve or poor ovarian response. We seek to determine whether these interventions can improve key ovarian parameters, including ovarian vascularization (measured by Resistance Index), ovarian reserve (assessed *via* Anti-Müllerian Hormone levels), follicular recruitment (evaluated by Antral Follicle Count), and follicular development (measured by follicle size).

PATIENTS AND METHODS

Prp 1ml/ovary. Neupogen 300mcg 0.5 ml/2 ovaries/

Study design

This study employed a prospective analytical design to evaluate the effects of Platelet-Rich Plasma, Granulocyte Colony-Stimulating Factor, and their combination (PRP + GSF) on ovarian function. The study included females who referred to the Infertility Department of Higher Institute for infertility Diagnosis and Assisted Reproductive Technologies, Baghdad, Iraq between July 2023 and December 2024. Participating females were prospectively enrolled and systematically followed from baseline through intervention and subsequent follow-up evaluations. They underwent cold ovarian drilling combined with either PRP, GSF, or a combination of both interventions, administered during the proliferative phase of their menstrual cycles (days 6–7). Ovarian parameters were prospectively measured at baseline and reassessed prospectively one to three months post-intervention, again on days 6–7 of the menstrual cycle.

Sample size

The sample size was calculated according to Andrew Fisher's Formula. The sample size was estimated to be a minimum of 80 participants by considering a 95% confidence level, a power of 80%.

sample size = (z score)^2x (std Dev)x (1 − std Dev)/(Confidence Interval) ^2

Patients

A total of 60 females were recruited for the study. The inclusion criteria required those diagnosed with diminished ovarian reserve or poor ovarian response based on clinical and laboratory assessments, between the ages of 25 and 45 years, and have regular menstrual cycles or irregular cycles due to ovarian insufficiency. Included females were excluded if they had autoimmune disorders, untreated endocrine disorders, or severe pelvic pathology. Additionally, those with a history of prior ovarian surgery or significant pelvic adhesions affecting ovarian function, as well as individuals who had used hormonal medications within the last three months before enrollment were not eligible for participation.

Randomization and group allocation

The included females (N=60) were randomly assigned into three equal groups (20 per group) using computer-generated random sequences for unbiased allocation. Group 1 (PRP-only) received Platelet-Rich Plasma injections into the ovaries, Group 2 (GSF-only) underwent Granulocyte Colony-Stimulating Factors therapy without PRP, and Group 3 (PRP + GSF) received a combination of PRP and GSF as an intervention.

Intervention procedures

Platelet-Rich Plasma was prepared by centrifuging blood samples to isolate PRP, which was then injected into the ovarian tissue under ultrasound guidance (1mL/ovary) for precise administration. Granulocyte Colony-Stimulating Factor were delivered *via* localized ovarian injections using Neupogen 300mcg divided to (0.25 mL in each ovary), following standardized dosage protocols to stimulate follicular recruitment and ovarian response. Participants in the combination therapy (PRP + GSF) group received both PRP and GSF injections sequentially.

Outcome measures and data collection

Ovarian function was assessed at two time points: baseline (pre-treatment assessment) and post-treatment

assessment (follow-up after the intervention period). Several key ovarian parameters were measured for each participant to evaluate the effects of the treatment. The Resistance Index (R.I.) was assessed using Doppler ultrasound to determine ovarian blood flow. Anti-Müllerian Hormone (AMH) levels were analyzed using an Enzyme-Linked Immunosorbent Assay (ELISA) to assess ovarian reserve. Antral Follicle Count (AFC) was evaluated through transvaginal ultrasound to determine the number of developing follicles, while follicle size was measured in millimeters using ultrasound imaging to track follicular development.

Ethical considerations

The study was approved by the Ethics Committee of High Institute for Infertility Diagnosis and Assisted Reproductive Technologies, Al- Nahrain University, Baghdad, Iraq (Code: 0701-PF-2020M26).

Data confidentiality

All participant data collected during this study will remain confidential and will only be used for research purposes. No personally identifiable information will be published or shared without the participant's explicit consent

Statistical analysis

Descriptive statistics, including mean, median, Standard Deviation (SD), and range, were calculated for all variables to summarize the data distribution. Normality testing was conducted using the Shapiro-Wilk test, where a p-value>0.05 indicated a normal distribution. For normally distributed variables, a paired samples t-test was applied to compare pre- and post-treatment values within each group. In cases where variables were not normally distributed, such as Anti-Müllerian Hormone (AMH), the Wilcoxon Signed-Rank Test was used. Statistical significance was set at a p-value of less than 0.05. All statistical analyses were performed using SPSS, with results presented in both descriptive and inferential statistical tables.

RESULTS

Effect of platelet-rich plasma on ovarian parameters

The first group included 20 participants. The mean age of participants was 35.18 years (SD=5.28), ranging from 27 to 43 years, with the Shapiro-Wilk test (W=0.918, p=0.137) indicating a normal distribution. The mean Resistance Index decreased from 0.604 (SD=0.105) before to 0.575 (SD=0.113) after treatment, with both distributions following normality (p>0.05). Anti-Müllerian Hormone levels increased from 0.950 (SD=0.618) to 1.065 (SD=0.713), with normality confirmed for both before (p=0.371) and after (p=0.388). Antral Follicle Count increased from 5.25 (SD=1.65) before to 5.85 (SD=2.35) after, with normal distribution confirmed (p=0.272 before, p=0.421 after). Follicle size also increased from 15.15 mm (SD=1.93) to 15.75 mm (SD=2.65), meeting normality assumptions (p=0.052 before, p=0.720 after) **(Tab. 1.).**

A paired samples t-test was conducted to evaluate the effect of Platelet-Rich Plasma therapy on various ovarian parameters, including Resistance Index, Anti-Müllerian Hormone, Antral Follicle Count, and follicle size. The results are summarized in **Tab. 2.** The mean R.I value before PRP therapy was significantly higher compared to post-treatment levels. The results of the paired samples t-test indicated a statistically significant decrease in R.I after PRP therapy, t (19)=2.282, p=0.034. This finding suggests that PRP may have contributed to improved ovarian vascularization, as indicated by the reduced resistance index. The comparison of AMH levels before and after PRP did not reveal a statistically significant difference, t(19)=-0.926, p=0.366. While a slight increase in mean AMH levels was observed post-treatment, the change was not significant, indicating that PRP did not have a measurable impact on AMH levels within the study period. The mean AFC value showed an increase following PRP therapy; however, the paired samples t-test revealed no statistically significant difference between pre- and post-treatment values, t (19)=-1.214, p=0.240. These results suggest that PRP did not significantly influence follicular recruitment during the observation period. A slight increase in follicle size was observed after PRP administration, but the change was not statistically significant, t d (19)=-1.189, p=0.249. This indicates that PRP therapy did not lead to a significant improvement in follicular growth.

Effect of granulocyte colony-stimulating factor on ovarian parameters

The second group included 16 participants, with four missing values, leading to the exclusion of age from further statistical analyses. The mean Resistance Index decreased from 0.620 (SD=0.0531) before GSF treatment to 0.592 (SD=0.0845) after, with normality confirmed (W=0.917, p=0.085 before; W=0.978, p=0.930 after). Anti-Müllerian Hormone levels increased from 1.096 (SD=0.5031) to 1.253 (SD=0.7954), with normality supported (W=0.941, p=0.437 before; W=0.950, p=0.568 after). Antral follicle counts improved from 4.500 (SD=1.6059) before treatment to 5.500 (SD=2.1398) after, with normal distribution confirmed (W=0.928, p=0.144 before; W=0.937, p=0.215 after). Follicle size increased from 15.200 mm (SD=1.7947) to 16.000 mm (SD=2.1026) following treatment, with normality verified (W=0.943, p=0.267 before; W=0.940, p=0.242 after) **(Tab. 3.).**

All ovarian parameters exhibited normally distributed data (p>0.05), justifying the use of parametric statistical analyses for further testing. A paired samples t-test was conducted to examine the impact of Granulocyte Colony-Stimulating Factor on key ovarian parameters, including Resistance Index, Anti-Müllerian Hormone, Antral Follicle Count, and follicle size. The statistical analysis compared pre- and post-treatment values to determine whether GSF administration led to significant changes in ovarian function. The results are summarized in **Tab. 4.**

The mean R.I. value decreased following GSF treatment, indicating potential improvement in ovarian vascularization. However, the statistical analysis revealed that this reduction was not statistically significant, t (17)=2.08, p=0.053. Although the p-value approached the conventional significance threshold of 0.05, the findings suggest that while GSF may contribute to enhanced blood flow, further investigation with a larger sample size is necessary to confirm its effect.

The analysis of AMH levels before and after GSF administration showed no significant difference, t (13)=-1.07, p=0.305. While AMH exhibited a slight increase

Tab. 1. Descriptive statistics and normality assessment of study variables before and after giving platelet rich plasma.

	Shapiro-Wilk							
	No.	**Missing**	**Mean ± SD**	**Median**	**Minimum**	**Maximum**	**W**	**p**
Age (Years)	17	3	35.176 ± 5.282	35	27	43	0.918	0.137
R.I (before)	20	0	0.604 ± 0.105	0.59	0.4	0.8	0.968	0.709
AMH (ng/mL)	20	0	0.95 ± 0.618	1.005	0.01	2	0.95	0.371
(before)								
AFC (before)	20	0	5.25 ± 1.65	5.5	2	8	0.943	0.272
Follicle size (mm)	20	0	15.15 ± 1.927	15	12	18	0.905	0.052
(before)								
R.I (after)	20	0	0.575 ± 0.113	0.565	0.4	0.8	0.941	0.246
AMH (ng/ mL)	20	0	1.065 ± 0.713	1.005	0.01	2.5	0.951	0.388
(after)								
AFC (after)	20	0	5.85 ± 2.346	6	2	10	0.953	0.421
Follicle size (mm) (after)	20	0	15.75 ± 2.653	16	11	21	0.968	0.72

Data presented as Mean ± SD (interquartile range) independent samples t-test
RI: Resistance Index, AMH: Anti Mullarian Hormone; AFC: Antral Follicle Count; W: Shapiro-Wilk Test Statistic, p: P-value

Tab. 2. Paired samples t-test results for pre- and post-treatment measurements before and after giving platelet rich plasma.

Paired Samples T-Test					
			statistic	**df**	**p**
R.I (before)	R.I (after)	Student's t	2.282	19	0.034
AMH (before)	AMH (after)	Student's t	-0.926	19	0.366
AFC (before)	AFC (after)	Student's t	-1.214	19	0.24
SIZE (before)	SIZE (after)	Student's t	-1.189	19	0.249

Note: H_a μ Measure 1 - Measure 2 ≠ 0
*: Data presented as Mean ± SD (interquartile range), independent samples t-test
RI: Resistance Index, AMH: Anti Mullarian Hormone; AFC: Antral Follicle Count; p: p-value; df: degrees of freedom; t: Student's t-test Statistic

Tab. 3. Descriptive statistics and normality assessment of study variables before and after giving growth stimulating factors.

Descriptives									
								Shapiro-Wilk	
	No.	**Missing**	**Mean**	**Median**	**SD**	**Minimum**	**Maximum**	**W**	**p**
age	16	4							
R.I (before)	20	0	0.62	0.61	0.0531	0.54	0.71	0.917	0.085
AMH (before)	14	6	1.096	1	0.5031	0.05	2	0.941	0.437
AFC (before)	20	0	4.5	4	1.6059	2	7	0.928	0.144
size (before)	20	0	15.2	15	1.7947	12	18	0.943	0.267
R.I (after)	18	2	0.592	0.59	0.0845	0.45	0.77	0.978	0.93
AMH (after)	14	6	1.253	1	0.7954	0.05	3	0.95	0.568
AFC (after)	20	0	5.5	6	2.1398	2	9	0.937	0.215
size (after)	20	0	16	16	2.1026	12	20	0.94	0.242

Data presented as Mean ± SD (interquartile range), independent samples t-test.
RI: Resistance Index; AMH: Anti mullarian hormone; AFC: Antral Follicle Count; SD: Standard Deviation; W: Shapiro-Wilk Test Statistic; p: p-value; df: degrees of freedom; t: Student's t-test Statistic

Tab. 4. Paired samples t-test results for pre- and post-treatment measurements before and after giving granulocyte colony-stimulating factor.

Paired Samples T-Test					
			statistic	**df**	**p**
R.I (before)	R.I (after)	Student's t	2.08	17	0.053
AMH (before)	AMH (after)	Student's t	-1.07	13	0.305
AFC (before)	AFC (after)	Student's t	-2.4	19	0.027
size (before)	size (after)	Student's t	-1.93	19	0.068

Note: H_a μ Measure 1 - Measure 2 ≠ 0
Data presented as Mean ± SD (interquartile range), independent samples t-test.
RI: Resistance Index; AMH: Anti Mullarian Hormone; AFC: Antral Follicle Count; p: p-value, df: degrees of freedom; t: Student's t-test Statistic

post-treatment, the observed change was not statistically meaningful. This result indicates that GSF may not have an immediate or substantial impact on ovarian reserve, as reflected by AMH levels. Future studies with extended follow-up periods may provide further insights into potential long-term effects.

A statistically significant increase in AFC was observed post-treatment, t (19)=-2.40, p=0.027, suggesting that GSF may enhance follicular recruitment. An increase in AFC is often indicative of improved ovarian response, which is relevant for reproductive function. These findings support the potential role of GSF in stimulating follicular development.

Follicle size increased following GSF administration; however, the change did not reach statistical significance, t(19)=-1.93, p=0.068. While the p-value suggests a possible trend toward larger follicle growth, the lack of statistical

significance implies that the observed difference may be due to random variation. A larger sample size or longer follow-up duration may be needed to determine whether GSF has a definitive impact on follicular growth.

Effect of granulocyte colony-stimulating factor and platelet-rich plasma combination on ovarian parameters

The third group included 20 participants with no missing values, with a mean age of 34.800 years (SD=7.1348), ranging from 19 to 43 years; however, the normality test (W=0.900, p=0.041) suggested that age was not normally distributed. The mean Resistance Index decreased from 0.588 (SD=0.0807) before treatment to 0.534 (SD=0.0811) after treatment, with normality confirmed (W=0.963, p=0.604 before; W=0.959, p=0.521 after). Anti-Müllerian Hormone levels declined from 4.800 (SD=13.6267) before treatment to 2.196 (SD=1.8857) after, with significant deviation from normality (W=0.322, p<0.001 before; W=0.632, p<0.001 after), indicating high variability. Antral Follicle Count increased from 4.950 (SD=1.9595) before treatment to 6.700 (SD=1.7199) after, with normality confirmed (W=0.935, p=0.189 before; W=0.933, p=0.174 after). Follicle size also increased from 14.575 mm (SD=2.2081) before treatment to 16.125 mm (SD=1.7462) after, with normality supported (W=0.963, p=0.610 before; W=0.913, p=0.074 after) **(Tab. 5.).**

All ovarian parameters, except AMH, exhibited normally distributed data (p>0.05), justifying the use of parametric statistical analyses for further testing. The high variability in AMH values suggests heterogeneous responses to GSF and PRP treatment, warranting further investigation.

A paired samples t-test was conducted to evaluate the effect of Granulocyte Colony-Stimulating Factor and Platelet-Rich Plasma on ovarian function, specifically analyzing changes in Resistance Index, Antral Follicle Count, and follicle size before and after treatment. The results are presented below. The results are summarized in **Tab. 6.**

Resistance index: A significant reduction in R.I. was observed following treatment, t(19)=4.50, p<0.001, indicating a statistically significant improvement in ovarian blood flow. The decrease in R.I. suggests that GSF and PRP may contribute to enhanced ovarian vascularization.

Antral follicle count: A statistically significant increase in AFC was noted post-treatment, t(19)=-3.52, p=0.002. This result suggests that GSF and PRP significantly improved follicular recruitment, which is indicative of enhanced ovarian responsiveness.

Follicle size: A significant increase in follicle size was observed after treatment, t(19)=-3.49, p=0.002. This finding suggests that GSF and PRP therapy may promote follicular development and maturation, leading to larger follicle sizes post-intervention.

A Wilcoxon signed-rank test was conducted to assess the effect of Granulocyte Colony-Stimulating Factor and Platelet-Rich Plasma on Anti-Müllerian Hormone levels before and after treatment. Given the non-normal distribution of AMH values, a non-parametric test was used instead of a paired t-test.

AMH Levels: The results indicated a statistically significant difference in AMH levels pre- and post-treatment, W=12.0, p=0.038. This suggests that GSF and PRP had a significant impact on AMH levels, potentially improving ovarian reserve. The results are summarized in **Tab. 7.**

Tab. 5. Descriptive statistics and normality assessment of study variables before and after giving growth stimulating factors and platelet rich plasma.

Descriptive									
								Shapiro-Wilk	
	No.	Missing	Mean	Median	SD	Minimum	Maximum	W	p
Age	20	0	34.8	37.5	7.1348	19	43	0.9	0.041
R.I (before)	20	0	0.588	0.585	0.0807	0.43	0.71	0.963	0.604
AMH (before)	20	0	4.8	1.25	13.6267	0.01	62.1	0.322	< .001
AFC (before)	20	0	4.95	5	1.9595	2	9	0.935	0.189
size (before)	20	0	14.575	14	2.2081	11	19	0.963	0.61
R.I (after)	20	0	0.534	0.515	0.0811	0.4	0.7	0.959	0.521
AMH (after)	20	0	2.196	2	1.8857	0.01	9.5	0.632	< .001
AFC (after)	20	0	6.7	6.5	1.7199	3	9	0.933	0.174
size (after)	20	0	16.125	16	1.7462	12	19	0.913	0.074

Data presented as Mean ± SD (interquartile range), independent samples t-test.
RI: Resistance Index; AMH: Anti Mullarian Hormone; AFC: Antral Follicle Count; SD: Standard Deviation; W: Shapiro-Wilk Test Statistic; p: p-value; t: Student's t-test Statistic

Tab. 6. Paired samples t-test results for pre- and post-treatment measurements before and after giving granulocyte colony-stimulating factor and platelet rich plasma.

Paired Samples T-Test					
			statistic	df	p
R.I (before)	R.I (after)	Student's t	4.5	19	< .001
AFC (before)	AFC (after)	Student's t	-3.52	19	0.002
size (before)	size (after)	Student's t	-3.49	19	0.002

Note: H_a μ Measure 1 - Measure 2 ≠ 0
P-value; df: Degrees of freedom, t: Student's t-test Statistic

Tab. 7. Wilcoxon signed-rank test for AMH before and after giving granulocyte colony-stimulating factor and platelet rich plasma.

Paired Samples T-Test				
			Statistic	p
AMH (before)	AMH (after)	Wilcoxon W	12[a]	0.038

Note: H_a μ Measure 1 - Measure 2 ≠ 0
[a] 8 pair(s) of values were tied

DISCUSSION

Ovarian reserve is the number of remaining eggs within the ovaries and is one of the factors used to evaluate fertility potential. Decreased ovarian reserve is the loss of the pool of follicles in the ovaries and affects fertility as well as the efficacy of the assisted reproductive technologies. Decreased ovarian reserve should be diagnosed accurately since it is associated with lower pregnancy rates as well as increased risk of miscarriage. The Bologna criteria, a widely used diagnostic approach, consider factors such as advanced maternal age (≥ 40 years), a history of poor ovarian response to stimulation (≤ 3 retrieved oocytes), and abnormal ovarian reserve markers (AMH 0.5–1.1 ng/ml, AFC 5–7 follicles). Reproductive medicine has also explored the use of new therapies like platelet-rich plasma and growth factor-stimulating factors as treatments of DOR. PRP therapy involves injecting growth factor-dense plasma straight into the ovary to stimulate the follicle, improve the flow of blood to the ovary, and reduce inflammation to potentially restore the functionality of the ovary. GSF treatments like the granulocyte colony-stimulating factor (G-CSF) stimulate the growth of the follicle and the health of the ovary through various molecular pathways.

Effect of platelet-rich plasma on ovarian parameters

The study evaluated the impact of Platelet-Rich Plasma therapy on various ovarian parameters, including Resistance Index, Anti-Müllerian Hormone levels, Antral Follicle Count, and follicle size in women with diminished ovarian reserve. The findings demonstrated a statistically significant decrease in R.I post-treatment, suggesting improved ovarian vascularization. Although increases in AMH levels, AFC, and follicle size were observed, these changes were not statistically significant.

Several studies have analyzed the effects of intraovarian PRP injection on ovarian function and fertility outcome. Éliás, et al. (2024) reported that a systematic review and meta-analysis demonstrated significant increases in the levels of AMH at 1, 2, and 3 months post-PRP treatment and also in AFC, indicating enhanced ovarian reserve. Wu, et al. (2024) also noted that PRP pre-treatment led to higher numbers of mature oocytes and transferable embryos in women with poor response of the ovaries to IVF [27]. Additionally, Fraidakis M, et al. noted in an observational study that intraovarian PRP injection stimulated the enhancement of the function and tissue of the ovaries as evidenced by significant changes in FSH and estradiol levels in women with diminished ovarian reserve [28]. The current study's findings align with existing literature, particularly regarding the improvement in ovarian vascularization post-PRP therapy. While other studies have reported significant enhancements in ovarian reserve markers and reproductive outcomes following PRP treatment, the lack of statistically significant changes in AMH levels, AFC, and follicle size in our study may be attributed to factors such as sample size, patient selection, or variations in PRP preparation and administration protocols.

Effect of granulocyte colony-stimulating factor on ovarian parameters

The study evaluated the impact of Granulocyte Colony-Stimulating Factor on ovarian parameters, including Resistance Index, Anti-Müllerian Hormone levels, Antral Follicle Count, and follicle size. The findings indicated a statistically significant increase in AFC post-treatment, suggesting enhanced follicular recruitment. Although decreases in R.I and increases in AMH levels and follicle size were observed, these changes did not reach statistical significance.

Similar studies have explored the effects of various growth factors on ovarian function. Administration of GH in conjunction with follicle-stimulating hormone has been shown to significantly increase estradiol secretion, indicating enhanced ovarian steroidogenic response. Notably, GH alone did not elicit a steroidogenic response, underscoring the synergistic effect when combined with FSH [29]. Supplementation with DHEA has been associated with improved ovarian response to stimulation, as reflected in the increase in the estradiol-to-gonadotropin dose ratio. This suggests that follicular development and oocyte quality may be enhanced by DHEA [30,31]. GDF9 is an oocyte-derived growth factor that has a critical role in early follicular development. Studies have demonstrated that GDF9 promotes the proliferation and differentiation of granulosa cells, which are essential for folliculogenesis. The observed significant increase in AFC following GSF treatment is in agreement with current studies demonstrating the positive effects of growth factors on follicular development. The non-significant changes in R.I, AMH levels, and follicle size may be attributed to factors such as sample size, treatment duration, or specific characteristics of the GSF used. Further research with larger cohorts and standardized protocols is necessary to fully elucidate the potential of GSF in enhancing ovarian function [29,32].

This study analyzed the cumulative influence of Granulocyte Colony-Stimulating Factor and Platelet-Rich Plasma on indicators of ovarian function including Resistance Index, Anti-Müllerian Hormone, Antral Follicle Count, and follicle size [33]. The findings suggest that GSF and PRP therapy may have a significant influence on the vascularization of the ovaries, recruitment of the follicles, and growth.

The study found a significant reduction in R.I. after treatment, indicating improved ovarian blood flow. A lower R.I. is associated with better ovarian perfusion, which may enhance follicular development by improving oxygen and nutrient delivery to the ovarian tissue [34]. This finding aligns with prior research indicating that PRP can promote angiogenesis, or the formation of new blood vessels, through the release of growth factors such as Vascular Endothelial Growth Factor (VEGF) and Platelet-Derived Growth Factor (PDGF), both of which contribute to ovarian tissue regeneration [14,35].

The increase in Antral Follicle Count confirms that GSF and PRP stimulated the recruitment of follicles. Increase in AFC is of utmost significance as it is generally correlated with improved fertility potential. Some studies have proven that the administration of PRP within the ovary is able to activate the latent follicles with subsequent increase in AFC, particularly in women with diminished ovarian reserve [36]. Additionally, growth factors such as Insulin-Like Growth Factor (IGF) and Epidermal Growth Factor (EGF) may also play a role in activating the process of folliculogenesis to aid the improvement in AFC [37,38].

A significant increase in follicle size was observed post-treatment, indicating improved follicular development and maturation. The observed follicle growth is consistent with reports that PRP and growth factors can enhance granulosa cell proliferation, which is essential for oocyte maturation [39,40]. Larger follicle sizes post-treatment suggest that the combination of PRP and GSF may enhance the growth of pre-antral and antral follicles, ultimately leading to improved ovulation potential [41,42].

The statistically significant change in AMH levels post-treatment suggests an impact on ovarian reserve. Interestingly, the mean AMH decreased from 4.800 to 2.196, indicating a possible variation in individual responses. While some studies report an increase in AMH levels post-PRP treatment due to improved follicular activation, others suggest that AMH fluctuations could be attributed to the dynamic nature of ovarian reserve markers or the heterogeneity of the patient population. The high variability in AMH values (SD=13.6267 pre-treatment, SD=1.8857 post-treatment) suggests that while some patients responded positively, others did not, highlighting the need for further investigation into personalized treatment approaches [14,43,44]

In summary Ovarian reserve, which refers to the number of remaining eggs in the ovaries, is a key determinant of fertility potential. Diminished ovarian reserve significantly affects fertility and the success of assisted reproductive technologies. Accurate diagnosis, often based on the Bologna criteria, is crucial due to its association with lower pregnancy rates and increased miscarriage risk. Recent advancements in reproductive medicine have explored platelet-rich plasma and growth-stimulating factors as potential therapies to enhance ovarian function.

LIMITATIONS

This study had several limitations. The sample size of 60 participants was relatively small, limiting the generalizability of the findings. Additionally, the short follow-up duration (1–3 months) may not reflect long-term treatment effects on ovarian function or fertility outcomes. There was also no control group receiving placebo or standard care alone, making it challenging to attribute observed changes exclusively to PRP or GSF interventions. Future research with larger sample sizes, longer follow-up periods, and inclusion of a placebo or no-intervention control group would strengthen the validity and applicability of these findings.

CONCLUSION

The combined use of Platelet-Rich Plasma and Growth Stimulating Factors significantly improved ovarian vascularization, follicular recruitment, and follicle growth. The reduction in Resistance Index suggests enhanced ovarian blood flow, while increased Antral Follicle Count and follicle size indicate improved ovarian responsiveness. Despite a significant change in Anti-Müllerian Hormone levels, the variability in responses highlights the complexity of ovarian reserve markers. While PRP and GSF show promise as therapeutic options for diminished ovarian reserve, further research is needed to standardize treatment protocols and assess long-term reproductive outcomes.

DATA AVAILABILITY

Data supporting the findings of this study are available upon reasonable request from the corresponding author.

AUTHOR CONTRIBUTIONS

Assistant professor Dr. Wasan Adnan Al-Jubori: Conceptualization and data curation. Assistant professor Dr. Muayad Sraibet Abbood: Formal analysis and data curation.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of Interest.

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