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Laparoscopic Intra-Ovarian Platelet Rich Plasma Injection for Ovarian Rejuvenation

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Abstract

Background: Ovarian failure, characterized by the loss of ovarian function, presents a significant challenge for women seeking to conceive. Conventional treatments have limitations in restoring ovarian function and fertility. Platelet-rich plasma (PRP) therapy has emerged as a potential regenerative approach for ovarian rejuvenation. The aim of this study was to assess the influence of intraovarian injection of PRP on ovarian function in a case with ovarian failure. Methods: This prospective interventional multi-center study included 50 women with primary and secondary ovarian failure selected from Obstetrics and Gynecology Department, Benha University Hospitals & other private centers. All patients were subjected to complete history taking, clinical examination, laboratory investigations including hormonal Laboratory evaluation of (FSH, LH, estradiol, AMH), PRP injection under intravenous anesthesia and post-operative follow up mainly for measuring ovarian hormones: FSH, LH, AMH, E2 every month for 6 months. Results: The current study included 50 patients. The mean age of them was (36.68 ± 6.36 years); range from (24 –45years). The median age (Q1, Q3) was 38 (32.75, 42). The mean serum follicle stimulating hormone (FSH) was 38.82 ± 20.38 (range 7.03 to 90.2) and its median (Q1, Q2) was 36.75 (19.75, 57). The mean serum luteinizing hormone (LH) was 21.43 ± 14.36 (range 4.3 to 65.5) and its median (Q1, Q2) was 16.9 (11.3, 28.7). The mean serum Estradiol hormone (E2) was 29.87 ± 22.72 (range 5 to 96) and its median (Q1, Q2) was 22.16 (13.1, 38.25). Conclusions: In the study group, intraovarian injection of PRP decreased FSH, LH, and increased E2 levels significantly, however it was less effective for boosting AMH levels. At 6-month intervals, the readings reverted to levels comparable to those that existed before to the PRP therapy. Therefore, PRP may be used alone or in conjunction with hormone therapy to treat infertility in women with poor ovarian reserve. In future clinical therapeutics, it might be regarded a time-efficient and cost-effective therapy technique.

Keywords: Laparoscopic; Intra-ovarian; Platelet Rich Plasma; Injection; Ovarian Rejuvenation.

1. Introduction

Ovarian failure, also known as premature ovarian insufficiency or early menopause, is characterised by the loss of ovarian function before the age of 40. It is a troubling prognosis for women, since it not only leads in the end of menstruation but also reduces childbearing potential [1]. Ovarian failure may be caused by a range of medical issues, including genetic abnormalities, autoimmune illnesses, chemotherapy, and radiation therapy. The illness affects around 1 percent of women globally and creates major reproductive health concerns [2, 3].

Hormone replacement therapy (HRT) has been the standard treatment for ovarian failure to reduce menopausal symptoms and avoid the long-term health hazards associated with oestrogen insufficiency [4, 5]. HRT may be beneficial for symptom management, but it does not treat the underlying cause of ovarian dysfunction or restore fertility. Consequently, innovative methods to enhance reproductive success and revitalise the ovary have gained popularity [6].

Platelet-rich plasma (PRP) treatment has emerged as a viable restorative technique in a number of medical specialties. Platelet-rich plasma (PRP) is obtained from the patient's blood and has a high concentration of platelets and growth factors [7]. It has been shown that these bioactive components possess angiogenic, anti-inflammatory, and tissue-regenerative effects. PRP has been applied effectively for the treatment of musculoskeletal problems, wound healing, and cosmetic operations [8].

In recent years, there has been increased interest in the possible use of PRP treatment in reproductive medicine, especially for ovarian rejuvenation. PRP's healing capabilities have motivated scientists to investigate its potential for boosting ovarian function and restoring fertility [9]. Intra-ovarian PRP injection includes injecting PRP directly into the ovaries in order to accelerate follicular development, increase ovarian blood flow, and perhaps rejuvenate the ovarian microenvironment [10].

Few research have examined the use of PRP for ovarian rejuvenation, and the available information is obtained mostly from animal studies and case reports [11].

Therefore, this study aimed to assess the effect of intraovarian injection of PRP on ovarian function in a case with ovarian failure.

2. Methods

This prospective interventional multi-center study included 50 women with primary and secondary ovarian failure who were selected from the Obstetrics and Gynecology Department at Benha University Hospitals and other private centers. The study was conducted between February 2021 and October 2022.

The study was conducted after receiving approval from the Benha University Faculty of Medicine's research ethics committee. All involved subjects gave their informed permission.

Inclusion criteria for the study were as follows: women between the ages of 20 and 45 years old, diagnosed with primary or secondary ovarian failure, body mass index (BMI) ranging from 19 to 29.

Exclusion criteria were cases of ovarian insufficiency caused by gonadal dysgenesis and chromosomal

abnormalities, the use of anticoagulants or steroids, NSAIDs presence of carcinomas, and active infections.

All participants underwent a comprehensive evaluation as part of the study protocol. Detailed history taking was performed, encompassing personal information, complaints related to infertility, medical history, obstetric history, menstrual history, contraceptive history, past medical problems, allergies, and family history of infertility or consanguinity.

A thorough clinical examination was conducted, including a general examination of vital signs and a local examination of the vulva, vagina, and cervix.

Routine laboratory investigations were carried out, including hormonal evaluations of follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol, and anti-Müllerian hormone (AMH). Additional laboratory tests, such as complete blood count, urinalysis, and random blood sugar, were performed as needed.

The preparation of platelet-rich plasma (PRP) followed strict aseptic techniques and temperature regulations. PRP was prepared according to the manufacturer's guidelines using a combination of anticoagulant (ACD) and blood collected under complete aseptic conditions. The PRP was then harvested and activated using calcium gluconate.

The laparoscopic procedure for PRP injection was performed under intravenous anesthesia. Two small incisions were made in the abdomen, and a laparoscope was used to inject 4ml of PRP into each ovary. The procedure was carried out using conventional techniques, and the needle was carefully guided into the ovarian cortex.

Postoperatively, patients were observed for six months period. Ovarian hormone levels, including FSH, LH, AMH, and E2, were measured monthly during this period.

Statistical analysis:

SPSS version 21 software was used to perform the statistical analysis (Spss Inc, Chicago, ILL Company). Using the Shapiro-Wilk test, the normality of the data distribution was determined. Nonparametric data were judged to be significant. The obtained data were summarized using mean Standard Deviation (SD) and range (minimum – maximum) for parametric numerical data, and median and interquartile range (IQR) (Q1, Q3) for non-parametric numerical data. The frequency and proportion were used to summarize qualitative data. Wilcoxon signed rank sum test (for non-parametric) was used to evaluate changes in parameters over two occasions, while Freidman's test was used to evaluate changes in parameters over more than two occurrences. A P-value with two tails less than 0.05 was judged statistically significant.

3. Results

The current study included 50 patients. The mean age of them was (36.68 ± 6.36 years); range from (24 – 45years). The median age (Q1, Q3) was 38 (32.75, 42). The mean serum follicle stimulating hormone (FSH) was 38.82 ± 20.38 (range 7.03 to 90.2) and its median (Q1, Q2) was 36.75 (19.75, 57). The mean serum leutinizing hormone (LH) was 21.43 ± 14.36 (range 4.3 to 65.5) and its median (Q1, Q2) was 16.9 (11.3, 28.7). The mean serum Estradiol hormone (E2) was 29.87 ± 22.72 (range 5 to 96) and its median (Q1, Q2) was 22.16 (13.1, 38.25). **Table 1**

Table (1) Demographics of the studied patients and Patients' hormonal profile estimated at first clinical attendance (n=50).

Variable		Frequency
Age (years)	Mean ±SD	36.68 ± 6.36
	Range (min. – max.)	24 - 45
	Median (Q1, Q2)	38 (32.75, 42)
Ovarian hormones	Baseline	
	Mean ±SD (range)	Median (Q1, Q3)
FSH	38.82 ± 20.38 (7.03 – 90.2)	36.75 (19.75, 57)
LH	21.43 ± 14.36 (4.3 – 65.5)	16.9 (11.3, 28.7)
AMH	0.07± 0.14 (0.01 - 0.97)	0.02 (0.014, 0.036)
Estradiol	29.87 ± 22.72 (5 - 96)	22.16 (13.1, 38.25)

The median baseline FSH level for the studied group was 36.75 and at different follow up times, FSH level significantly decreased across time ($P^1< 0.001$). The lowest level for FSH was by the 5th month (11.25). The FSH at the 6th month was 12.2 which significantly lower than the baseline level ($p^2 =0.008$). The median baseline LH level for the studied group was 16.9 and at different follow up times, LH level significantly decreased across time ($P^1< 0.001$). The lowest level for LH was in the 6th month (6.9). The LH in the 6th month was significantly lower than the baseline level ($p^2 =0.008$). **Table 2**

Table (2) Comparison between FSH and LH at different periods (n=50)

Variable		Median (Q1, Q3)	Test statistics	P value
FSH	**Baseline**	36.75 (19.75, 57)	49.9	**P^1 < 0.001** **Post hoc test** **P^2 = 0.008**
	1st month	25.8 (10.6, 37.1)		
	2nd month	22.45 (10.65, 34.2)		
	3rd month	17.6 (10.1, 22.8)		
	4th month	13.2 (8.7, 26.6)		
	5th month	11.25 (8.3, 30.7)		
	6th month	12.2 (6.8, 20.8)		
LH	**Baseline**	16.9 (11.3, 28.7)	28.7	**P^1 < 0.001** **Post hoc test** **P^2 = 0.008**
	1st month	11.5 (6.7, 19.9)		
	2nd month	11.8 (5.4, 17.3)		
	3rd month	8.7 (4.7, 17.8)		
	4th month	9.1 (3.3, 17.9)		
	5th month	7.2 (4.3, 13.7)		
	6th month	6.9 (3.8, 10.9)		

P^1, comparison of repeated measures across all time points using **Friedman's test** (equivalent test to repeated measures ANOVA test). P^2 comparison between admission and 6th month, using **Wilcoxon signed ranks test.**

The median baseline AMH level for the studied group was 0.02 that significantly increased in the 1st month to 0.40 (p^2 = 0.001) then significantly decreased across 2nd, 3rd, 4th, 5th and 6th months to 0.30, 0.18, 0.09, 0.04 respectively (P^1=0.023). The level for AMH by the 6th month was equal to its level at baseline with no significant difference (p^3 = 0.066). The median baseline E2 level for the studied group was 22.16 and at different follow up times, E2 level significantly increased across time (P^1=0.004). The highest level for E2 was in the 6th month (44). The E2 in the 6th month was significantly higher than the baseline level (p^2 =0.015). **Table 3**

Table (3) Comparison between AMH and E2 at different periods (n=50)

Variable		Median (Q1, Q3)	Test statistics	P value
AMH	**Baseline**	0.02 (0.014, 0.36)	14.6	**P^1 =0.023** **Post hoc test** **P^2 = 0.001** P^3 = 0.066
	1st month	0.40 (0.28, 0.72)		
	2nd month	0.30 (0.21, 0.54)		
	3rd month	0.18 (0.126, 0.324)		
	4th month	0.09 (0.063, 0.162)		
	5th month	0.04 (0.028, 0.072)		
	6th month	0.02 (0.14, 0.036)		
E2	**Baseline**	22.16 (13.1, 38.25)	6	**P^1 =0.004** **Post hoc test** **P^2 = 0.015**
	1st month	33.9 (14.7, 46)		
	2nd month	32.6 (21.75, 47.8)		
	3rd month	34.5 (23.33, 50.25)		
	4th month	32.52 (19.1, 54.25)		
	5th month	33 (22.65, 58.45)		
	6th month	44 (24.3, 51.8)		

P^1, comparison of repeated measures across all time points using **Friedman's test** (equivalent test to repeated measures ANOVA test). P^2 comparison between admission and 1st month, using **Wilcoxon signed ranks test.** P^3 comparison between admission and 6th month, using **Wilcoxon signed ranks test.**

The current study 24% of the studied patients were ovulated and 76% of them were non ovulated.

According to spontaneous pregnancy 6% of the studied patients continued till delivery but 8% of them reported abortion. Regarding ICSI 4% continued till delivery and 6% failed. **Table 4**

Table 4: Outcome of PRP injection among the study group.

Variable		Frequency No. (%)
Spontaneous	**Continued till delivery**	3 (6%)
	Abortion	4 (8%)
ICSI	**Continued till delivery**	2 (4%)
	Failed	3 (6%)

4. Discussion

In the current study, the patients mean age was (36.68 ± 6.36 years); range from (24 –45years), median age (Q1, Q3) was 38 (32.75, 42).

In line with our research, a prospective controlled trial conducted by **(12)** investigated the potential of intraovarian injections of autologous PRP to rejuvenate the ovaries and reactivate folliculogenesis in women experiencing early ovarian insufficiency. The study included 50 infertile women with precocious ovarian insufficiency. Results showed that the mean age of the participants was 31.1 years (±4.38 SD) within a range of 24 to 38 years. The average duration of infertility was 2.66 years (±1.33 SD) ranging from 1 to 5 years, and the average BMI was 31.11 kg/m2 (±3.48 SD) with a range of 25 to 37.6 kg/m2. Among the participants, 39 (78%) had primary infertility, while 11 (22%) had secondary infertility [12].

Regarding serum follicle stimulating hormone (FSH) and serum leutinizing hormone (LH) in the current work. In line with our findings, **(12)** reported that the mean serum follicle stimulating hormone (FSH) was 40.51 ± 15.61 (mIU/ml), serum leutinizing hormone (LH) has a mean value of 19.43 ± 13.36 IU/mL, serum Estradiol hormone (E2) had a mean value of 28.04 ± 12.31 (pg/mL) [12] [12].

Regarding our findings, the median baseline FSH level for the studied group was 36.75 and at different follow up times, FSH level significantly decreased across time (P1< 0.001). The lowest level for FSH was in the 5th month (11.25). The FSH in the 6th month was 12.2 which was significantly lower than the baseline level (p2 =0.008). Consistently, **(12)** declared that FSH level had significantly decreased across time. The lowest level for FSH was in the 3rd month. The FSH in the 3rd month was significantly lower than the baseline level (P-value <0.001) [12].

In an intriguing finding, **(13)** observed a notable decrease in FSH levels (UI/ml) during the second menstrual cycle (7.05±1.43) compared to the first cycle (8.30±2.13), showing a significant difference with a P-value of less than 0.001 when compared to the FSH level prior to PRP therapy (11.50±4.05). However, after a six-month period following the therapy, the FSH level (11.28±3.23) had returned to the pre-treatment levels [13].

In terms of LH at different periods, the median baseline LH level for the studied group was 16.9 and at different follow up times, LH level significantly decreased across time (P1< 0.001). The lowest level for LH was in the 6th month (6.9). The LH in the 6th month was significantly lower than the baseline level (p2 =0.008). Consistent with the findings of **(12)**, our study also demonstrated a statistically significant decrease in estradiol levels across different periods (P-value < 0.001) [12]. In line with our results **(13)** observed a similar trend in LH (UI/ml) levels. The difference between the first and second menstrual cycles (5.10±1.29 and 5.20±1.44, respectively) was comparable, and the subsequent recovery to pre-PRP levels (pre-PRP, 7.25±1.92 and at 6 months, 6.00±2.36) was less pronounced [13].

In the current study, the median baseline AMH level for the studied group was 0.02 that significantly increased in the 1st month to 0.40 (p2 = 0.001) then significantly decreased across 2nd, 3rd, 4th, 5th and 6th months to 0.30, 0.18, 0.09, 0.04 respectively (P1=0.023). The level for AMH by the 6th month was equal to its level at baseline with no significant difference (p3 = 0.066). Conforming our results, **(12)** found that AMH varied statistically significantly at different periods (P-value = 0.041), it was 0.09 ± 0.39 at baseline, then increased in the first and second months (0.1 ± 0.63, 0.12 ± 0.71, respectively), then decreased again by the third month to 0.11 ± 0.8 [12]. Our findings align with the results reported by **(13)**, demonstrating that the AMH level (ng/ml) increased after PRP treatment in both the first (0.82±0.33) and second menstrual cycle (0.99±0.36), with a significant increase observed in the second cycle (P<0.05). However, by the 6th month following treatment (0.71±0.33), the AMH level significantly decreased (P<0.05) compared to the level in the second menstrual cycle post-treatment. Although it remained slightly higher than the pre-PRP treatment level (0.69±0.32), the difference was not considered significant [13].

In the present work, the median baseline E2 level for the studied group was 22.16 and at different follow up times, E2 level had significantly increased across time (P1=0.004). The highest level for E2 was in the 6th month (44). The E2 in the 6th month was significantly higher than the baseline level (p2 =0.015). Parallel to our results, **(12)** showed that the difference in estradiol at different periods was statistically significant as its level had significantly increased across time (P-value < 0.001) [12]. Our findings are consistent with the findings of **(13)**, who observed a significant increase of approximately 50% in the level of estradiol. Contrary to our findings, **(14)** documented a consistent increase in estradiol levels from the 1st to the 6th month after PRP treatment. Subsequently, there was a slight decrease at 12 months. They also reported that the most significant levels of estradiol were observed at the 6th and 12th

months compared to pre-rejuvenation levels ($p < 0.0003$; $p < 0.00005$) [14].

In contrast to our findings, **(15)** conducted a study on women with primary ovarian insufficiency and found that intra-ovarian injection of autologous PRP had no significant impact on FSH levels. They also observed only minimal improvement in AMH levels [15]. In line with our findings, **(16)** reported that there was no significant difference in the hormonal profile, specifically LH and FSH levels, among women with poor ovarian response (POR) or primary ovarian insufficiency after receiving PRP injection [16].

5. Conclusion

In conclusion, intraovarian injection of PRP was beneficial for boosting FSH, LH, and E2 levels in the study group, but less effective for increasing AMH levels. At 6-month intervals, the readings reverted to levels comparable to those before to the PRP treatment. Therefore, PRP may be used alone or in conjunction with hormone therapy to treat infertility in women with diminished ovarian reserve. Future clinical treatments may consider it a cost-effective and time-consuming therapy technique. Prior to practical application, however, the safety and efficacy of this novel treatment method, as well as its short- and long-term adverse effects, must be investigated in further high-quality research.

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