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Evaluation of the impact of platelet-rich plasma in women with reduced ovarian reserve

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ABSTRACT

Introduction: Infertility is the most critical factor disrupting the marital relationship, which imposes high financial and psychological costs on couples. Despite vast advances, the problem of infertility has not yet been entirely resolved. The new method of injecting platelet-rich plasma (PRP) has been promising for couples. This study investigated the effect of PRP injection on the fertility of infertile women.

Material and Method: In this study, 40 women with a history of infertility with a mean age of 37.75 were included in the study. Prior to the demographic information intervention, laboratory findings, including serum anti-mullerian hormone (AMH) levels and ultrasound for the number of antral follicles count (AFC) were performed. Autologous PRP was then prepared for each patient, and an intraovarian injection was performed. Two months after injection, serum levels of AMH and AFC levels were re-evaluated.

Results: The mean AMH levels before and after the injection were 0.07±0.05 and 0.13±0.06, respectively ($p<0.001$). The mean follicle-stimulating hormone (FSH) levels before and after the injection were 33.95±12.59 and 22.10±3.29, respectively ($p<0.001$). The amount of AFC increase one number after the injection. Linear regression showed that patient and partner age, body mass index (BMI) and infertility years had no effect on PRP injection response. PRP is associated with a significant increase in AMH levels, decrease in FSH levels and a significant increase in the number of AFCs. Patient and partner age, BMI and infertility years have no effect on response to PRP injection.

Conclusion: This study showed that the PRP injection improving ovarian reserve markers as measured by AMH and FSH serum levels, and AFC. However, further studies should be done to assess the effect of PRP on pregnancy outcomes.

Keywords: Infertility, platelet-rich plasma, follicle-stimulating hormone, antral follicle count , anti mullerian hormone

INTRODUCTION

Infertility has become a prevalent issue, which about 15% of couples have faced. There are several possible causes, 40% of which are related to women, 40% to men, and 20% to both couples (1). Disorder of the ovulation process is one of the most common causes of infertility in women (2). Particularly, the referral of women with poor ovarian reserves (POR) to infertility centers has increased in recent years due to a change of priorities in young girls and their marriage at older ages (3). Female age is largely the main factor limiting the success of spontaneous fertilization and assisted reproductive technology (ART), which is mainly due to the loss of ovarian follicle reserve and ovule quality as women age increases, especially after the age of 37 (4-6). Approximately 12.7- 35.5 % of infertility cases in women are related to ovulation disorders (7). In cases it is the only cause of infertility, there is a highly satisfactory response to adjuvant treatment. These individuals' successful treatment is dependent on careful examination and discovery of the main cause of anovulation. Ovulation stimulation is a pharmacotherapy used in women without regular ovulation with ovulation disorders, or polycystic ovary syndrome (PCOS). These pharmacotherapies are performed to increase the number and quality of ovules to increase the chance of fertility and the success rate of ART (8). The drugs used for this purpose are called ovulation-stimulating drugs, which can be used to increase the number and quality of ovules. To use ART, one of the

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measures which are first taken is ovulation stimulation to obtain the proper number of the high-quality oocyte. Although the natural cycle can also be used in women with normal ovarian function to prepare oocytes, since these treatments require time and money, controlled ovarian stimulation should be used to increase the chance of fertility. To stimulate ovulation in ART treatments, it is better to avoid ovarian overstimulation as far as possible to prevent potential risks while obtaining a suitable number of the oocyte. Therefore, the person should be carefully examined before any treatment (9).

One of the new treatments for the controlled ovarian stimulation for the treatment of women with reduced ovarian reserve is the platelet-rich plasma (PRP) injection. PRP has been widely used and successfully in different fields such as orthopedics, wound healing, hair growth promotion, ophthalmology, and wound regeneration, and its application is increasing (10). PRP has now become a field of interest in reproductive medicine with higher focus on infertility. PRP is used in research fields such as poor ovarian reserve, menopause, premature ovarian failure, and thin endometrium (11-13). PRP increases local delivery of cytokines, growth factors ,lysosomes and chemokines and also,modifies the inflammatory responses,cell proliferation and cell differentiation (11).

In this study, the effect of PRP injection on ovarian function has been investigated. Mean serum level of anti-müllerian hormone (AMH) and follicle-stimulating hormone (FSH) levels before and after PRP injection and the number of antral follicle count (AFC) in this study have been measured and ovarian function was measured on its basis. The main aim is to evaluate the success rate of the PRP injection method and to evaluate the factors affecting the success rate.

MATERIAL AND METHOD

The study was carried out with the permission of the Clinical Research Ethics Committee of Gümüşhane University. (Date: 09/06/2021, Decision No: 2021/4) All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki. Fourty participants in this study visited Medical Park Samsun Hospital and BAU Göztepe Medical Park Hospital for infertility treatment from January 2020 to January 2021. Participants ranged in age from 23 to 40 years.

Inclusion and Exclusion Criteria

The inclusion criteria were the age of women between 25-45 years with POR, and the duration of infertility was more than one-year. POR diagnosis was made by using POSEIDON criteria. The exclusion criteria were malignancy history, previous abdominal surgeries, and anticoagulant use.

Laboratory Parameters

PRP is taken from a venous blood sample and injected into the ovary after preparation. PRP activates factors such as VEGE and TGF, which are effective in VEGE migration and extracellular matrix accumulation.

PRP preparation

We prepared PRP using a T-lab autologous platelet-rich plasma kit (T-Biotechnology Laboratory, Bursa, Turkey). The preparation process was based on the manufacturer's instructions (24). Under sterile conditions, 20 ml of blood samples were taken from each patient, and then the samples were centrifuged in 830 g tubes for eight minutes. A 5 ml syringe with a 16G needle was used to receive PRP. About 2 to 4 cc of PRP from the first tube and about 4 to 8 cc of PRP from the second tube were collected without removing the growth factor-rich blood clot. After PRP collection, the resulting solution was transferred to a separate tube and then gently shaken for thirty to sixty seconds.

Intraovarian Injection

Two hours after PRP preparation and under conscious sedation, the intraovarian injection was performed in the operating room. We used a 17 G 35 cm single lumen needle (Cook, USA) and ultrasound guidance to inject PRP transvaginally into at least one ovary. Older patients with POR usually have small, fibrotic ovaries; In these cases, new planes in the ovary were created by distention and injection at various sites, and PRP injection was performed in this way. After the operation, the patients were transferred to the recovery room, and after 40 minutes of monitoring, they were discharged.

Statistical Analysis

Data were analyzed, tabulated, and subjected to using the SPSS (version 26). The continuous data were displayed as mean±SD. At the same time, categorical data were illustrated as percentages and numbers. The Kolmogorov-Smirnov test of normality was utilized to test the normality hypothesis. Based on the test results, proper parametric (Paired-samples t-test) and nonparametric tests (Wilcoxon signed-rank test) were used. A p-value of <0.05 was regarded as statistically significant.

RESULTS

A total of 40 women (mean age±SD: 37.75±4.46) with the diagnosis of POR were included in the study. The participants' BMI and partner age were 23.72±2.51, and 39.10±3.76, respectively. The mean duration of infertility is 6.17±2.08 years. **Table 1** shows the explanatory information of the variables. Descriptive characteristics of other variables omit for brevity.

Table 1. Explanatory information of the variables

Variable	N	Minimum	Maximum	Mean	SD
Patient age (yr)	40	25.00	45.00	37.75	4.46
Partner age(yr)	40	33.00	45.00	39.10	3.76
Body mass index	40	21.00	30.00	23.72	2.51
Infertility duration (yr)	40	3.00	12.00	6.17	2.08

A paired-samples t-test was conducted to compare AMH scores in an infertile woman before and after PRP injection. There was a significant difference in the scores for Pre-AMH (M=0.07, SD=0.05) and Post-AMH (M=0.13, SD=0.06) conditions; t(39)=-7.804, p=0.000.

A Wilcoxon signed-rank test showed that PRP injection did a statistically significant change in FSH scores in infertile woman (Z=-5.481, p=0.000). There was a significant difference in the scores for Pre-FSH (M=33.95, SD=12.59) and Post-FSH (M=22.10, SD=3.29).

A Wilcoxon signed-rank test was conducted to compare the quantity of AFC in an infertile woman before and after PRP injection. There was a significant difference in the quantity for Pre-AFC (M=1) and Post-AFC (M=2). **Table 2** shows the significant difference in variables before and after PRP injection **Figure 1** shows PRP treatment resulted in lower serum FSH, higher serum AMH, and higher AFC. Linear regression showed that patient and partner age, BMI and infertility years had no effect on PRP injection response in terms of serum level of AMH. **Table 3** shows linear regression analysis predicting serum AMH difference.

Table 2. The significant difference in variables before and after PRP injection

Variables	Pre PRP Injection		Post PRP Injection		t(39)/Z	p
	Mean	SD	Mean	SD		
FSH	33.95	12.59	22.10	3.29	-5.481	0.000*
AMH	0.07	0.05	0.13	0.06	-7.804	0.000**
AFC	1	0	2	0	-6.325	0.000*

*Wilcoxon signed-rank test, **Paired-samples t-test

Table 3. Linear regression analysis predicting serum AMH difference

Variables	B	SE	Beta (β)	t	p
Patient age	-0.003	0.002	-0.301	-1.226	0.228
Partner age	0.001	0.003	0.058	0.233	0.817
Body mass index	0.000	0.003	-0.022	-0.132	0.896
Duration of infertility (years)	0.005	0.004	0.236	1.392	0.173

Note: Constant=0.121, F(4,40=1.206), p<.001, RP=.121

DISCUSSION

This study dealt with the effect of PRP injection on FSH, AMH, and AFC. Our findings showed improved ovarian reserve markers such as FSH, AMH, and AFC with a 2-month treatment course with PRP. In addition, there was an association between the use of PRP and significantly increased biochemical variables such as AMH. Our findings showed that none of the factors such as age, BMI, and infertility duration affect the effect of the PRP method on improving ovarian function.

The first study was performed on the effect of PRP on ovarian reserve with Sills et al. (14) in 2018 with four patients. After PRP injection, an improvement in ovarian reserve was observed. All four patients showed decreased

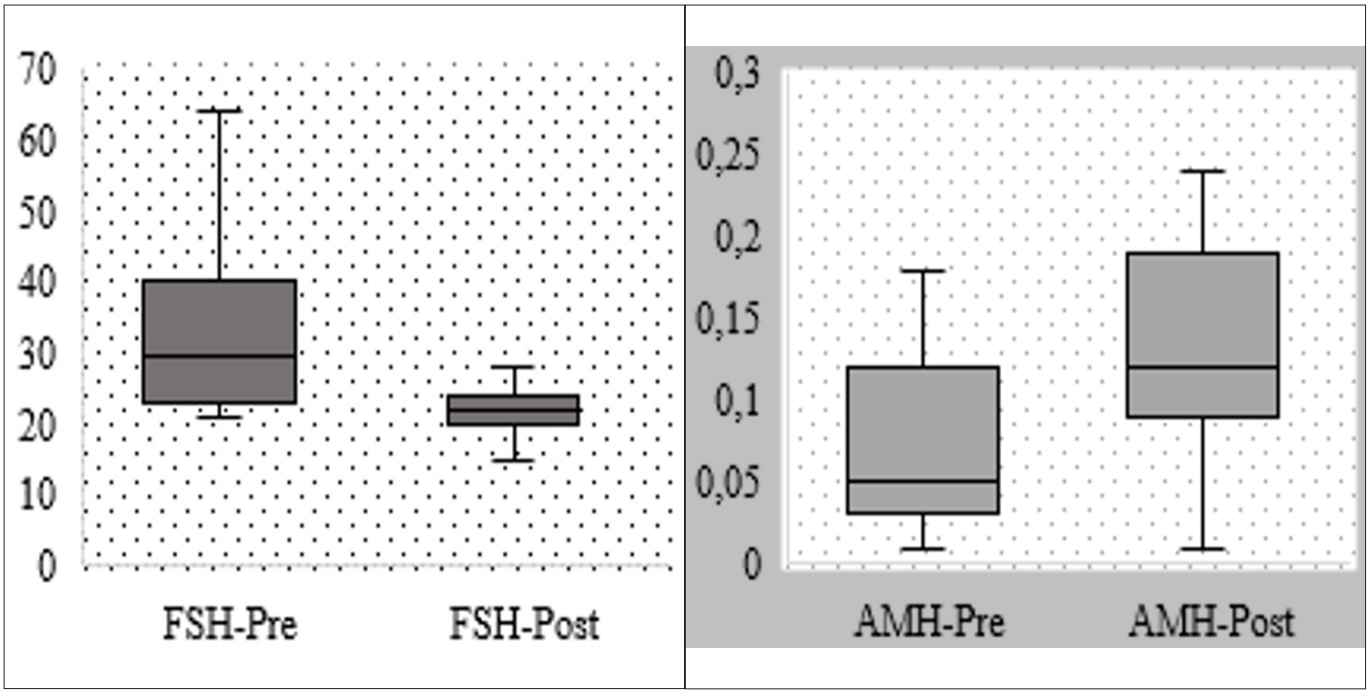

Figure 1. AMH and FSH levels before and after PRP injection.

FSH. Three of the four patients showed an increase in AMH. In 2020, Sills measured the previous research question in another study, expanding the number of samples to 182. Measurement of FSH and AMH levels in two-week intervals found that PRP injection had a significant effect on improving ovarian function. The most frequent positive effect has been reported after 4 weeks (15).

Sfakianoudis et al. (16) reported improved ovarian function in women with POR after PRP injection. Three studied patients reported an increase in AMH and a decrease in FSH after three months of PRP injection. Sfakianoudis et al. (17) in another study reported reduced cycle cancellation rates after PRP treatment.

In a study, Pacu et al. (18) measured the effect of PRP on 20 women with POR. Based on findings, the PRP injection decreased the FSH, luteinizing hormone (LH), and estradiol (E2) values and increased the AMH. The number of canceled cycles has also decreased significantly. Six months after PRP injection, the ovarian function has returned to the pre-injection period.

In a study with 510 women with POR, Cakiroglu et al. (19) focused on improving ovarian function after PRP injections. This study is of special value among similar studies due to its large sample size. Based on the findings, the PRP injection significantly increases AMH and significantly decreases FSH.

Melo et al. (20) reported an improved ovarian function in the case group after PRP injection comparing ovarian function in the case and control groups. In 46 women with POR in the case group, a significant improvement was reported compared to the control group with 37 women in ovarian function, including increased AMH and decreased FSH .

Other studies performed with three patients and with one patient emphasized the significant effect of PRP injection on the ovarian function of the studied patients (21,22).

The effect of PRP injection on the fertility rate has also attracted the attention of researchers. In the conducted studies, the PRP injection has been reported on fertility rates in spontaneous and ART methods (19-20,23,24). However, the effect of PRP injection on increasing fertility and live birth rates requires further studies with larger sample size.

Improved ovarian function is not affected by factors such as female age, partner's age, infertility duration, and BMI due to the effect of PRP injection. Based on our study findings, female age does not affect the probability of success of the PRP injection method as a determining success factor of the ART. Infertility duration, partner's age, and BMI also affect improved ovarian function.

CONCLUSION

This study showed that the PRP injection is secure. This improves ovarian reserve markers measured by FSH and AMH serum levels and antral follicle count. However, further studies should be done to assess the effect of PRP on outcomes of pregnancy among women undergoing ART.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Clinical Research Ethics Committee of Gümüşhane University (Date: 09/06/2021, Decision No: 2021/4)
Informed Consent: All patients signed the free and informed consent form.
Referee Evaluation Process: Externally peer-reviewed.
Conflict of Interest Statement: The author has no conflicts of interest to declare.
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Author Contributions: The author declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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