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Effect of intraovarian platelet rich plasma injection during hysterectomy on ovarian reserve and sexual life

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ABSTRACT

Objectives: Low ovarian reserve is a major concern as older women seek pregnancy. Women 35 and older who want to conceive have fewer high-quality oocytes, which is linked to a drop in Anti-Müllerian hormone (AMH). Women in this age bracket may need hysterectomy for abnormal uterine bleeding, leiomyomas, and pre-/malignant reasons. Those with preserved ovaries after hysterectomy nevertheless experience menopause. This circumstance impacts women's sexuality. Interventions to improve ovarian function in this age group may illuminate both concerns.

Material and methods: We did an observational case-control study of 35–50-year-old individuals who had hysterectomy for a benign disease. The trial group received perioperative PRP for their ovaries, while the control group received no treatment. Patients' preoperative AMH and Female Sexual Function Index (FSFI) scores were compared to those 3 months following surgery.

Results: Our study found a substantial drop in postoperative AMH levels in the control group ($p < 0.001$), whereas the PRP group showed a slight increase in AMH levels. The PRP group has a significant difference in preoperative and postoperative AMH levels ($p = 0.0001$).

The PRP group had a much higher total FSFI score. Subgroup analysis shows this variation in lubrication, orgasm, contentment, and pain. The PRP group had substantial differences in orgasm, pleasure, and pain pre- and post-operation.

Conclusions: PRP supports ovarian regeneration but has limited benefits. This information should be used to build standardized PRP protocols and undertake more extensive investigations by applying PRP to numerous organs, including the ovaries.

Keywords: PRP; AMH; FSFI; fertility; menopause

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INTRODUCTION

As women grow older the number of oocytes decreases, and the premenopausal period begins between the ages of 40 and 50 years old. Menopause occurs when there is a significant decrease in the number of oocytes and progressive atresia in the oocytes at an average age of 51 years old. In the early menopausal period, a decrease in the number of follicles is associated with a decline in inhibin B and Anti-Müllerian hormone (AMH) concentrations, and Follicle-stimulating hormone (FSH) levels begin to rise.

At the present, the increasing demand for fertility at advanced ages and the support provided by the assisted reproductive technique make pregnancy possible in this critical time interval between the premenopausal and the initiation of the menopausal periods. As a matter of fact, the majority of patients in *in vitro* fertilization (IVF) programs are those with low ovarian reserve after male factor and unexplained infertility, conversely the success rate of IVF in this group with older patients is lower compared to younger patients [1, 2].

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Adding to the hormonal climate, patients in this age group may be candidates for hysterectomy due to abnormal uterine bleeding, uterine fibroids and/or pre-malignant causes. Although hysterectomy does not necessarily result in surgical menopause when the ovaries are preserved, it is observed that it alters the ovarian functions [3, 4]. Although, the effectual mechanism is not yet fully understood, it is linked to impaired ovarian blood flow [5, 6]. Therefore, in patients undergoing hysterectomy in the premenopausal period, menopausal symptoms are often observed, and accompanying sexual dysfunctions may also occur.

Platelet rich plasma (PRP) contains unidentified growth factors and bioactive agents that contribute to tissue regeneration through its proliferative and anabolic cell response [7]. Its use is common in orthopedics, sports medicine, dermatology, and andrology [8–10]. Although some studies in the literature report positive results with intraovarian PRP, its clinical benefits are debated [11].

Anti-Mullerian hormone (AMH) is a biochemical marker secreted from preantral follicles. It is used to predict ovarian reserve due to being an indicator of the primordial follicle pool. It can be measured on any day of the menstrual cycle. As the primordial follicle pool decreases with age, AMH levels also start to decline [12].

It also known that sexual function decreases with hormonal changes and premenopausal symptoms [13, 14]. Sexual function is best described by patient's self-reporting and scored by the Female Sexual Function Index (FSFI) [15]. The FSFI is translated and validated into several languages, allowing its use in different countries and communities.

We hypothesized that the PRP will have impact on the ovarian reserve with local response translating into increase or maintenance of the AMH serum levels and in the improvement of the patients' sexual life.

Our primary objective was to evaluate the effect of the intraovarian injection of PRP on the serum levels of AMH. The secondary objective was to assess the impact of the intraovarian injection of PRP in the sexual life of premenopausal women that received hysterectomy.

MATERIAL AND METHODS

We conducted an observational case-control study in patients that underwent hysterectomy in the age range of 35 to 50 years of age because of a benign condition. Patients' clinical files were retrospectively reviewed, and data extracted on the variables of interest. Preoperative variables included: age, marital status, body mass index (BMI), co-morbidity, indications for hysterectomy and AMH. Sexual life was assessed by the FSFI Turkish validated questionnaire [16]. Hysterectomy was performed laparoscopically or by abdominal incision and included as variable due to potential impact in the FSFI.

We excluded patients under the age of 35 or over 50 years old, those who underwent cystectomy simultaneously with hysterectomy, patients with endometriosis, and those with a final pathology of malignancy. Patients that could not fill out the FSFI questionary because their native language was not Turkish, or illiteracy were also excluded.

Measurements

Levels of AMH were determined in the treatment and the control groups in the morning of the operation. During operation the intervention group received an intraovarian injection of PRP material as a single dose of 2 mL per ovary. No intervention was performed on the ovaries of the control group of patients. After operation AMH levels were determined in both groups at 3rd month.

Sexual function was evaluated in control and treatment groups by using the Turkish validated version of the FSFI at baseline and then 3 months after hysterectomy [16]. FSFI consists of 19 questions assessing 6 items: sexual desire, arousal, lubrication, orgasm, satisfaction, and pain parameters, enabling the evaluation of female sexual functions. To score the FSFI, the sum of each domain score is first multiplied by a domain factor ratio. The domain factor ratio is 0.6 for desire, 0.3 for arousal and lubrication, 0.4 for orgasm,

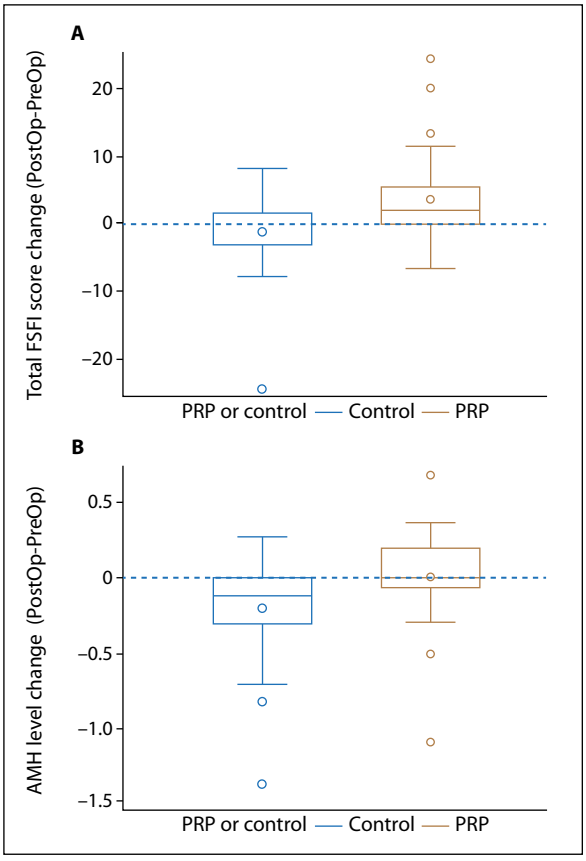


Figure 1. Total Female Sexual Function Index (FSFI) score change (**A**); Anti-Müllerian hormone (AMH) level change (**B**); PRP — platelet rich plasma

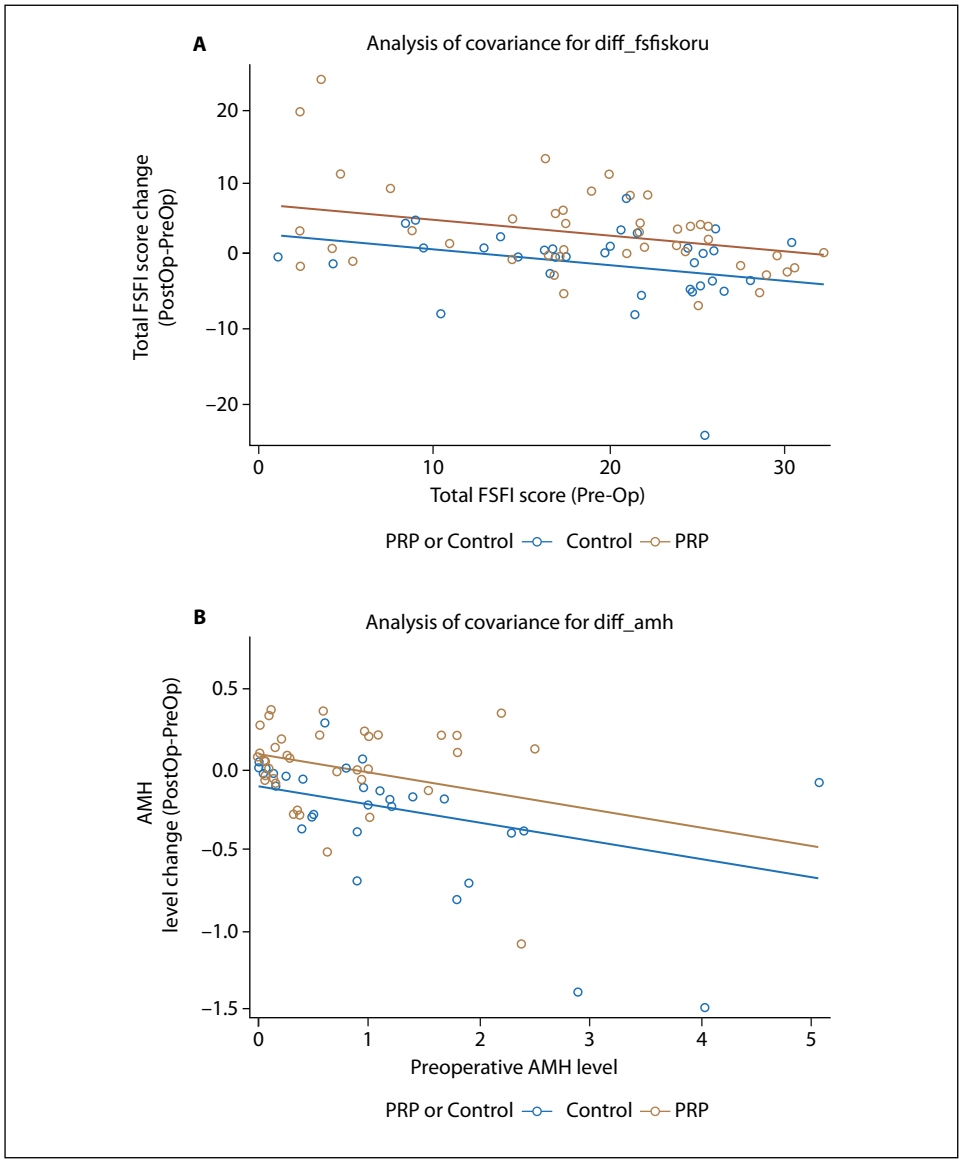


Figure 2. Analysis of covariance for difference between baseline total Female Sexual Function Index (FSFI) score and total FSFI score change (**A**); Analysis of covariance for difference between baseline Anti-Müllerian hormone (AMH) level and AMH level change (**B**); PRP — platelet rich plasma

satisfaction, and pain. The scores below 26.55 are clinically relevant to sexual dysfunction (specificity 73.3%; sensitivity 88.9%) [17]. Maximum score is 36 and minimum score is 2 or 1.2 as satisfaction domain can have 0.8 or 0 point as minimum score [18]. In the questionnaire that we used in our study the minimum score was 1.2.

Patients were instructed on the different dimensions and questions of the FSFI and asked to self-report. In case of doubt, they could provide a trained nurse that would clarify the question. Female Sexual Function Index (FSFI) questionnaire was filled up a week before operation and at 3 months of follow-up coinciding with the AMH determination.

PRP preparation method

In our study, blood was drawn into two T-LAB PRP tubes through routine phlebotomy using a vacuum during the surgery. Each of them contained 8 mL blood. PRP was prepared using the T-LAB PRP kit®, a class 2B certified device. During the preparation procedure, centrifugation was applied twice for 2 minutes at 500 G using the T-LAB 415P® centrifuge device. The upper and middle portions are then subjected to centrifugation again after the first centrifugation. Platelet rich plasma (PRP) was ready after the buffy coat (middle layer) was taken. As the PRP was used immediately, we didn't have to store it.

PRP injection method

After the hysterectomy procedure completed the long needle in the T-LAB PRP kit® was inserted *via* the suprapubic route. Platelet rich plasma (PRP) material was applied as a single dose of 2 mL per ovary at 6 points, each 6 mm in depth, with the long needle. Six injection sites were selected in the ovarian cortex and superficial stroma, targeting evenly distributed locations across the ovary to enhance uniform delivery of PRP. The intraovarian injection procedure is illustrated in Figure 3.

Statistical analysis

Categorical variables were presented as frequencies and percentages, and continuous variables were presented as mean and standard deviation. To compare the distribution of a continuous variable between the PRP and controls, Wilcoxon–Mann–Whitney test will be used. To assess the association between a categorical factor with PRP categorization, Chi-square or Fisher's exact tests will be used as appropriate. To assess the impact of the baseline measurements on the change of the scores and its interaction with the PRP categorization, we used analysis of covariance (ANCOVA) models.

All analyses were conducted using SAS® Version 9.4 (Cary, North Carolina, USA). Imputation was planned in case of missing data in the FSFI responses. All the questionnaires are filled out and all the questions are completed.

Ethical approval was obtained from the Human Research Ethics Committee of our hospital in December 2020 (Approval number: 173). The study was compliant with the Helsinki Declaration principles for human research. Informed consent was obtained from all patients.

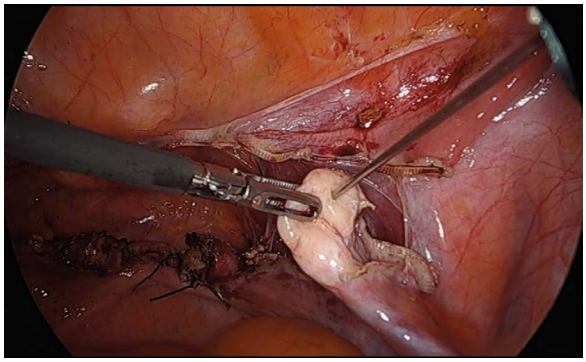


Figure 3. View of platelet rich plasma (PRP) injection into the ovarian cortex using a long needle through the suprapubic route

RESULTS

From January 2021 to January 2022, 81 consecutive patients were included in the study, 35 (43.2%) in the control group and 46 (56.8%) in the PRP group. In Table 1, we present the baseline characteristics of the two groups.

No comorbidities were found in 17 (48.6%) and 25 (54.3%) of the patients in the control and PRP groups respectively. The most common comorbidity was hypertension and diabetes mellitus in both groups. Two individuals had rheumatoid arthritis, and one had epilepsy, classified as comorbidities among others.

The most common indications for hysterectomy were uterine myomas (37.1–39.1%) and abnormal uterine bleeding

Table 1. Baseline characteristics of the patients categorized by groups

Variables	All (n = 81)	Control group (n = 35)	PRP group (n = 46)
Age (median, IQR)	43 (36–48)	43 (38–48)	43.5 (36–48)
BMI (mean, SD)	30.15 ± 5.38	30.12 ± 5.39	30.12 ± 5.39
Marital status (n, %)			
Single	8 (9.9)	3 (8.6)	5 (10.9)
Married	73 (90.1)	32 (91.4)	41(89.1)
Comorbidity (n, %)			
Hypertension	12 (14.8)	6 (17.1)	6 (13.0)
DM	10 (12.3)	4 (11.4)	6 (13.0)
Hypothyroidism	7 (8.6)	4 (11.4)	3 (6.5)
Hypertension and DM	7 (8.6)	3 (8.6)	4 (8.7)
Other	3 (3.7)	1 (2.9)	2 (4.3)
Indication hysterectomy (n, %)			
Uterine myoma	31 (38.3)	13 (37.1)	18 (39.1)
Abnormal uterine bleeding	35 (43.2)	13 (37.1)	22 (47.8)
Adenomyosis	6 (7.4)	2 (5.7)	4 (8.7)
Other	9 (11.1)	7 (20.0)	2 (4.3)

DM — diabetes mellitus; IQR — interquartile range; PRP — platelet rich plasma; SD — standard deviation

Table 2. Differentiation of Female Sexual Function Index (FSFI) items and Anti-Müllerian hormone (AMH) (mean, ± SD)

		Preoperative			Postoperative			Differentiation between pre-post		
		Mean	SD	p value	Mean	SD	p value	Mean	SD	p value
FSFI desire score	All	4.49	1.75	0.26	4.74	1.94	0.0091	0.25	1.74	0.44
	Control	4.31	1.95		4.17	1.99		−0.14	1.70	
	PRP	4.63	1.60		5.17	1.80		0.54	1.73	
FSFI arousal score	All	8.41	4.46	0.95	10.99	5.19	0.062	6.49	4.77	0.13
	Control	8.37	4.17		9.97	5.52		5.66	5.03	
	PRP	8.43	4.71		11.76	4.85		7.13	4.51	
FSFI lubrication score	All	10.12	5.77	0.61	10.79	5.38	0.12	0.67	4.21	0.06
	Control	10.60	5.28		9.89	5.35		−0.71	3.40	
	PRP	9.76	6.15		11.48	5.35		1.72	4.49	
FSFI orgasm score	All	8.05	4.45	0.075	8.70	4.57	0.054	0.65	3.46	0.013*
	Control	8.31	3.87		7.74	4.09		−0.57	2.55	
	PRP	7.85	4.87		9.43	4.82		1.59	3.79	
FSFI satisfaction score	All	8.49	4.80	0.40	8.78	4.93	0.28	0.28	3.52	0.016*
	Control	9.14	4.34		8.17	4.73		−0.97	3.44	
	PRP	8.00	5.12		9.24	5.07		1.24	3.31	
FSFI pain score	All	8.10	4.95	0.53	8.20	4.76	0.27	0.10	3.18	0.0068*
	Control	8.60	4.34		7.54	4.20		−1.06	2.83	
	PRP	7.72	5.39		8.70	5.12		0.98	3.18	
Total FSFI score	All	18.11	8.50	0.68	19.65	8.77	0.054	1.54	6.06	0.0061*
	Control	18.70	7.73		17.84	8.35		−0.86	5.39	
	PRP	17.66	9.11		21.02	8.92		3.36	5.95	
AMH level	All	0.75	0.88	0.22	0.67	0.81	0.95	-0.08	0.31	0.0001*

*p < 0.05 is considered statistically significant; PRP — platelet rich plasma; SD — standard deviation

(37.1%–47.8%) in the control and PRP groups. Other indications for hysterectomy included focal endometrial neoplasia and simple endometrial hyperplasia without atypia.

Overall, a history of previous abdominal surgery other than cesarean section was observed in 9 (25.7%) and 12 (26.1%) in the control and the PRP group, respectively.

There were neither statistically significant differences in baseline characteristics between the control and PRP injection group nor in terms of the surgical approach to hysterectomy (Fisher's p value = 0.23), with 69% of women receiving a laparoscopy hysterectomy.

In our study, when comparing baseline AMH levels with postoperative AMH levels, a significant decrease is observed postoperatively in the control group ($p < 0.001$), whereas in the PRP group, although not significant, there is an increase in AMH levels. When evaluating the difference between preoperative and postoperative AMH levels, a significant difference ($p = 0.0001$) was observed favoring the PRP group (mean 0.02 SD 0.27) over the control group (mean −0.21 SD 0.31) (Tab. 2).

Also, when the control and PRP groups were examined, the total FSFI score was significantly higher in the PRP group (Tab. 2). When the difference between pre- and post-operation was examined, orgasm, satisfaction, and pain scores were significantly improved in the PRP group. While desire and arousal scores increased modestly, the changes were not statistically significant. In contrast, the control group showed minimal or negative changes in these domains (Tab. 2).

By constructing, analysis of covariance (ANCOVA) models, we also investigated whether the baseline measurements of these scores have significant impact on the change from preoperative to postoperative and whether this association is modified by the PRP intervention. The interaction term was not significant in the difference of orgasm, satisfaction, pain or total FSFI score and not for AMH. Nevertheless, the baseline FSFI score is positively correlated with the postoperative increase, similarly, the baseline AMH value is negatively correlated with the postoperative decrease.

DISCUSSION

In our study, we targeted premenopausal patients both as potential candidates to IVF because of the low ovarian reserve and sexual dysfunctions due to premenopausal processes that may significantly affect the quality of life in this age group. Our data shows that intraovarian PRP is at least effective in maintaining AMH serum levels at three months after injection. When intragroup comparison was made, AMH levels decreased significantly in the control group, while AMH levels were preserved in patients who received PRP. Furthermore, the total FSFI score increased significantly in the PRP injection group after the intervention.

Diminished ovarian reserve (DOR) is particularly challenging for fertility specialists, so alternative methods are being studied and applied to increase low ovarian reserve. One of these is the use of androgen supplementation before ovarian stimulation. In a study aiming to increase ovarian response, 80 mg/day oral micronized DHEA was given to patients for 2 months before recombinant FSH treatment for intrauterine semination, which resulted in enhanced ovarian responsiveness and reduced gonadotropin requirements [19, 20].

That kind of application has a wide portfolio in terms of side effect profiles and, they are only promising during IVF applications. No side effects, including allergic side effects, were observed in any of our 46 patients with PRP applications.

In an intraovarian PRP study conducted by Hosseinisadat et al. [21] in 22 infertile patients who were planned for IVF due to low ovarian response, a significant increase in AMH was detected in the patients. Procedure was similar to our study except they injected PRP transvaginal. They also compared the levels of AMH before and three months after the procedure. But the major difference is that the patients had GnRH antagonist protocol before PRP injection. As this protocol may influence the results, we attribute the obvious increase in AMH to the younger patient age group, the small patient population.

In addition, although the possibility of spontaneous pregnancy in DOR patients is quite rare, in a retrospective cohort study of 507 patients, the spontaneous pregnancy rate in POI patients under hormone replacement treatment (HRT) was reported as 3.6% [22]. In the study conducted by Çakiroglu et al. [23], spontaneous pregnancy was observed in 23 (7.4%) of 311 patients when IVF treatments were planned after intraovarian PRP [23]. In this respect, PRP promises to be used not only as an additional treatment method in IVF patients, but also as it increases the possibility of spontaneous pregnancy.

Quality of life (QoL), although based on person's subjective evaluation and influenced by multiple factors it is one of the basic indicators on the success and acceptability of a medical intervention in a modern society. Evaluation of general QoL is complex, whith sexual life being and important dimension for which specific tools have been developed [24].

Besides secondary infertility due to low ovarian reserve, another significant problem due to the premenopausal patient group is genital atrophy. Vaginal burning during coitus and decrease sexual desire, lubrication and arousal are not infrequent during this period with almost 70% of women reporting a disruption in the sexual dysfunction when the FSFI questionnaire is used with the lowest score described for sexual desire [25–27]. Recent studies have also reported positive effects of PRP application in genitourinary atrophy, indicating potential benefits on vaginal health and sexual function beyond hormonal mechanisms [28].

In our study, the total FSFI score increased significantly in the PRP group after the operation. When the differences between pre- and post-intervention were assesed separately for each domain desire and lubrication decreased in the control group and increased in PRP group although the difference was not significant and arousal improved slightly in both groups, again not in a statistically significant way. Nonetheless, the difference in score improvement was statistically significant for orgasm, satisfaction and pain leading to a global better sexual function in those women that received PRP injection. As frequently occurs in structured QoL evaluations the increase in FSFI was greater in those women with higher scores at baseline [29].

Our study is not devoid of limitations. Despite no significant differences observed in our study in any of the pre-intervention variables between control and PRP groups, the lack of randomization implies selection bias. Selection for PRP injection was driven by the current legislation in our country that authorizes the treatment only below a determined serum cut-off level of AMH. We do acknowledge that the size of the cohorts was small and may have masked possible differences in outcomes; however, it was designed as pilot study based on a rational hypothesis. At the same time, the limited sample size precluded propensity matching for inverse probability treatment weighted (IPTW) analysis and thus the effect of the variable intervention (PRP) may be still uncertain. Additionally, patients were not blinded for the intervention leading to a potential bias in FSFI assessment and results. Lastly, although the manufacturer's instructions to obtain the PRP material were thoroughly followed we cannot completely rule out that the double centrifugation of the serum may reduce the reliability of the method and thus needs validation.

According to a systematic review and meta-analysis, PRP demonstrates better effects in increasing AMH levels after 2 months of intervention [30]. In a recent systematic review, most of the studies measured the outcomes 2–3 months after the intraovarian injection of PRP [31]. Consequently, to mitigate the impact of surgery on FSFI and to allow for ovarian recovery, we planned a three-month follow-up.

CONCLUSIONS

Inspite of the limitations above, we believe that the findings of our study will be a ray of hope for patients with low ovarian reserve. As a result of this study and subsequent similar studies, if an increase in the AMH values of patients with low ovarian reserve is observed, the number of applications for assisted reproductive techniques will decrease and the possibility of spontaneous pregnancy will increase. As a result, both medical costs and the negative psychological effects on patients during treatment will decrease. In addition, if the need for assisted reproductive techniques continues, PRP application will provide additional support to the treatments.

Based on the findings of this study, PRP can be applied to patients with endometriosis, a condition that gynecologists are particularly wary of, to prevent ovarian damage caused by the surgery.

The effect of the application method and location on the results should be studied. Thus, if ease of application is improved for patients who experience sexual dysfunction due to intense menopausal symptoms in the premenopausal period, PRP can take its place in routine treatment protocols for these patients.

In conclusion, intraovarian PRP may be a promising adjunct to improve ovarian function and sexual quality of life in selected premenopausal women.

Article information and declarations

Data availability statement

Authors have all the data. They can share it if necessary.

Ethics statement

Ethical approval was obtained from the Human Research Ethics Committee of our hospital in December 2020 (Approval number: 173).

Author contributions

ABAD — design, data collection, writing, ÖA: design, data collection; PÖ — design, data collection, EB: data collection, writing; MK — statistical analysis, supervision; ÇK — data collection, supervision.

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Conflict of interest

All authors declare no conflict of interest.

Supplementary material

None.

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