J Surg Med. 2020;4(12):1195-1200.
DOI: 10.28982/josam.824815

Research article
Araştırma makalesi

Journal of Surgery and Medicine
e-ISSN=2602-2079

An experimental study on the long-term and short-term effects of PRP treatment on the endometrium and ovaries

PRP tedavisinin endometriyum ve yumurtalıklar üzerindeki uzun vadeli ve kısa vadeli etkileri üzerine deneysel bir çalışma

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Ethics Committee Approval: Sakarya University Medical Experimental Application and Research Center (SUDETAM), Ethics Committee (Number: 05.02.2020/14) approved the study.
Etik Kurul Onayı: Sakarya Üniversitesi Tıbbi Deneysel Uygulama ve Araştırma Merkez (tarihi ve numarası: 05.02.2020/14) çalışmayı onayladı.

Conflict of Interest: No conflict of interest was declared by the authors.
Çıkar Çatışması: Yazarlar çıkar çatışması bildirmemişlerdir.

Financial Disclosure: The authors declared that this study has received no financial support.
Finansal Destek: Yazarlar bu çalışma için finansal destek almadıklarını beyan etmişlerdir.

Published: 12/30/2020
Yayın Tarihi: 30.12.2020

Abstract

Aim: It has been reported that ovaries have stem cells, and they can be used in the treatment of patients with low ovarian reserves by maturing them through some growth factors. PRP, a new therapy, has many bioactive compounds including growth factors. This study aimed to investigate the long and short-term biochemical and histopathological effects of platelet-rich plasma (PRP) treatments on the lining of the uterus and the ovaries in rats.

Methods: Female rats (n = 21) were randomly divided into 3 groups: A sham group, a short-term PRP group and a long-term PRP group. At the end of the study, anti-Mullerian hormone (AMH) and follicle-stimulating hormone (FSH) levels were examined in blood samples obtained from the intracardiac area, and the ovaries and uterus of the rats were examined to determine the histopathological and immunohistochemical findings.

Results: The results of this study demonstrated that, in the blood samples of the rats, the AMH levels increased and FSH levels decreased in the short-term PRP group ($P<0.05$). When the uteri and ovaries of the rats were examined histopathologically, positive and beneficial effects of the PRP therapy were found in the short-term PRP group. However, considering the long-term results, the positive effects of PRP decrease as time goes on.

Conclusion: This study showed that PRP application delivers beneficial effects to the uterus and ovaries in female rats in the short term.
Keywords: Anti-Mullerian hormone, Follicle-stimulating hormone, Ovary, Platelet-rich plasma, Rat, Endometrium

Öz

Amaç: Yumurtalıkların kök hücrelere sahip olduğu ve yumurtalık rezervi düşük olan hastaların bazı büyüme faktörleri ile olgunlaştırılarak tedavisinde kullanılabileceği bildirilmiştir. Yeni bir tedavi olan PRP, büyüme faktörleri dahil olmak üzere birçok biyoaktif bileşiğe sahiptir. Bu çalışma, sıçanlarda trombositten zengin plazma (PRP) tedavilerinin uterus ve yumurtalıkların iç yüzeyine uzun ve kısa vadeli biyokimyasal ve histopatolojik etkilerini araştırmayı amaçladı.

Yöntemler: Dişi sıçanlar (n=21) rastgele 3 gruba ayrıldı: Sham grup, kısa süreli bir PRP grubu ve bir uzun süreli PRP grubu. Çalışma sonunda intrakardiyak alandan alınan kan örneklerinde anti-Mullerian hormonu (AMH) ve folikül stimüle edici hormon (FSH) seviyeleri incelendi ve ratların yumurtalık ve uterusu incelendiğinde histopatolojik ve immünohistokimyasal bulgular.

Bulgular: Sıçanların kan örneklerinde kısa süreli PRP grubunda AMH düzeylerinin arttığını ve FSH düzeylerinin düştüğünü göstermiştir ($P<0.05$). Sıçanların rahim ve yumurtalıkları histopatolojik olarak incelendiğinde, kısa süreli PRP grubunda PRP tedavisinin olumlu ve faydalı etkileri tespit edildi. Ancak uzun vadeli sonuçlar dikkate alındığında PRP'nin olumlu etkileri zaman geçtikçe azalmaktadır.

Sonuç: PRP uygulamasının kısa vadede dişi sıçanlarda rahim ve yumurtalıklara faydalı etkiler sağladığını göstermiştir.
Anahtar kelimeler: Anti-Mullerian hormone, Follicle-stimulating hormone, Over, Platelet-Rich plasma, Rat, Endometriyum

How to cite/Atıf için: Budak Ö, Toprak V, Turkler C. An experimental study on the long-term and short-term effects of PRP treatment on the endometrium and ovaries. J Surg Med. 2020;4(12):1195-1200.

Introduction

It is thought that the number of female reproductive cells is fixed at birth and exhausted over time due to increasing age, natural periods, and external factors such as toxins [1]. In some studies, it has been reported that ovaries have stem cells and that they can be used in the treatment of patients with low ovarian reserves by maturing them with some growth factors [2].

Platelet-rich plasma (PRP), a new therapy, is used in many health fields (surgery, dermatology, orthopedics, and dentistry) [3, 4]. PRP is defined as the autologous concentration of platelets: 3 to 5 times more than the physiological concentration of platelets in normal blood [5]. PRP has a therapeutic effect due to the presence of various factors, such as transforming growth factor β (TGF-β), fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), insulin-like growth factor 1 (IGF-1), platelet-derived growth factor (PDGF), connective tissue growth factor (CTGF), zinc (Zn) and superoxide dismutase (SOD). The beneficial effects of PRP arise mainly from these many bioactive compounds [6].

In experimental studies in rats, PRP has also been shown to protect the ovaries in ovarian ischemia-reperfusion injury [7]. It has been observed that the growth of the primordial germ cell line is due to the growth factors contained in PRP, which induce their proliferation in in vitro culture mediums [8]. It has been reported that in many studies involving menopausal patients, the ovarian reserves are insufficient, follicle-stimulating hormone (FSH] levels decrease, and that the application of intraovarian PRP can increase oocyte production [2,9,10]. There is no conclusion about how long the positive effect of PRP continues in these studies.

Also, the long- and short-term effects of PRP on the uterus and ovaries have not been reported in published literature yet. The aim of this study was to investigate the long- and short-term biochemical and histopathological effects of PRP therapy applied to rat uteri and ovaries.

Materials and methods

Animals

Experimental animals were obtained from the Sakarya University Medical Experimental Application and Research Center (SUDETAM). A total of twenty-one Sprague-Dawley female rats weighing 235 ± 20 grams were randomly selected for use in the experiments. The rats were all 3 months old, sexually active and had a menstrual cycle. Animals were housed and fed at normal room temperature (22 to 24°C) prior to the experiment. The study was conducted at the Sakarya University Experimental Studies and Research Center. The experimental procedure was approved by the Sakarya University Committee for Animal Research. The recommendations of the 'The European Commission Directive 86/609/ECC guidelines' were taken into consideration. (Ethics Committee Number: (05.02.2020/14).

PRP preparation method

The blood samples were taken from the tail veins of the rats to prepare for PRP therapy. These blood samples from rats were placed into vacuum tubes containing 0.1 M of citrate buffer. PRP was prepared using the two-stage centrifuge method. The final concentration of the PRPs obtained was confirmed to be 6.2 × 106 platelets/ml and was made ready for application [11].

Experimental groups and procedures

Rats were randomly divided into three groups before the experiment as follows: The short-term PRP group, the long-term PRP group and a control group scheduled for a sham operation. The Sprague-Dawley female rats had a menstrual cycle 6 times in one month and all rats completed the study without any fatalities.

Sham group (SG; n = 7): A single dose of 0.9% saline (1 ml/kg) was administered to the uterus and ovaries of the rats in this group; the rats were sacrificed by decapitation after 3 menstrual cycles.

Short-term PRP group (ST-PRP; n = 7): A single dose of PRP (1 ml/kg) was administered to the uterus and ovaries of the rats; the rats were sacrificed by decapitation after 3 menstrual cycles.

Long-term PRP group (LT-PRP; n = 7): The rats with a single dose of PRP (1 ml/kg) to the uterus and ovaries were kept for 3 months. At the age of 6 months (after 18 menstrual cycles), the rats were sacrificed by decapitation to examine the long-term effects of PRP on the ovaries and endometrium [12, 13].

All surgical procedures were carried out under sterile conditions in proper laboratory settings. All rats were intraperitoneally administered 200/10 mg/kg (i.p.) of ketamine/xylazine for anesthesia and then the uterus and ovaries were observed through a 2 to 2.5 cm incision in the lower abdomen. PRP was injected into the uterus and ovaries of the rats in the ST-PRP and LT-PRP groups. At the same time, the same volume of 0.9% saline was injected into the SG group. The abdomens of all groups were sutured after the procedure. The rats in the SG and ST-PRP groups were sacrificed by decapitation after 3 menstrual cycles. The rats in the LT-PRP groups were sacrificed by decapitation after 18 menstrual cycles.

When the groups were sacrificed, blood was taken intracardially under anesthesia for anti-Mullerian hormone (AMH) and FSH measurement. Their uterine and ovarian tissues were removed, and tissue samples were evaluated histopathologically. The biochemical, immunohistochemical (IHC) and histopathological results were compared between the three groups.

Biochemical analysis of AMH and FSH

Under anesthesia, intracardiac blood samples were taken from rats with the help of an injector and transferred into glass tubes. These samples were centrifuged at 3000 rpm for 10 minutes and their serum was separated. FSH and AMH levels in the serum samples were measured by the Roche Modular Analytics system E170 electrochemiluminescence immunoassay method. Serum hormone levels were measured with an autoanalyzer (Beckman Coulter DXI-600). The results were expressed as nanograms/milliliter (ng/ml).

Histopathological analysis

A histological examination was performed at the histology department in the medical faculty of Sakarya University. After removal of the uterus and ovaries, samples were fixed in 10% formaldehyde. The tissues were passed through a 50%, 60%, 70%, 80%, 90%, 96% and 100%

concentrated ethanol series, respectively. The tissues were firstly embedded in paraffin and then cut into 4 µm-thick sections, which were deparaffinized with xylene and rehydrated with alcohol and water. Sections were stained with hematoxylin-eosin (HE) and Masson-Trichrome (MT) (According to the BioOptica application procedure, Milan, ITALY). Tissue sections examined under a microscope (Nikon Eclipse Ni-inverted microscope, Nikon Corp., Japan) with a digital camera system (NIS Elements Imaging Software, Nikon Corp., Japan) by a histologist under blind conditions.

The histological findings in the uterine tissue, including fibrosis, vascular proliferation and inflammation (neutrophil infiltration), were evaluated. Semi-quantitatively, a scoring scale from 0 to 3 was used. The amount of fibrosis was scored as follows:

0 = no fibrosis

1 = minimal fibrosis

2 = moderate fibrosis, and

3 = dense fibrosis.

Inflammation was scored as follows:

0 = no inflammation

1 = occasional lymphocytes and plasma cells

2 = presence of plasma cells, eosinophils and neutrophils

3 = presence of many inflammatory cells.

Vascular proliferation was scored as follows:

0 = no vascular proliferation

1 = mild vascular proliferation

2 = moderate vascular proliferation, and

3 = intense vascular proliferation [14].

2.6 Immunohistochemical examination

Ki-67 (GeneTex; Cat. No: GTX16667; USA) and VEGF (GeneTex; Cat. No: GTX102643; USA) were used to demonstrate tissue stimulation and proliferation in the endometrium and ovaries. Sections of 5 µm were taken from the tissue samples. The prepared slides were incubated

values were used for the descriptive statistical analysis. The differences between with VEGF (1: 400) and Ki-67 (1: 400) for 1 hour at room temperature in a humid environment. At the end of the incubation period, the slides were washed with Phosphate Buffered Saline (PBS). The slides were then stained with diaminobenzidine (DAB) and HE for 10 minutes.

For the Ki-67 and VEFG immunoreactivity assessment in uterine tissue, a light microscope was used at 200× magnification. Ki-67 was analyzed semi-quantitatively in the immuno-staining assessment by selecting ten random areas on the stained slide. One hundred cells were selected and displayed from each area. Ki-67 indices were expressed as the percentage of cells stained positively compared to all cells [15,16]. To assess VEGF expression, the number of capillaries and proliferation cells were counted, and counting was done by selecting at least 3 random areas [17].

For the Ki-67 and VEFG immunoreactivity assessment in ovarian tissue, a light microscope was used at 200× magnification. For Ki-67, positive cells were randomly counted in five different areas. The VEGF was randomly counted in 10 areas and the scoring system used was 1 = weak, 2 = medium and 3 = strong [18].

Statistical analysis

SPSS software version 22.0 was used to conduct the statistical analysis (SPSS Inc., Chicago, IL). Mean and standard deviation the groups were assessed by One-Way ANOVA (Tukey analysis). A *P*-value of less than 0.05 was considered statistically significant.

Results

The blood hormone results of this study are summarized in Table 1. The AMH level in the blood was 1 (0.14) ng/ml in the SG and 1.5 (0.33) ng/ml in the ST-PRP. When compared with SG, the increase in AMH level in ST-PRP was significant ($P<0.05$). However, sufficient AMH elevation was not detected in the LT-PRP group.

Short-term PRP application significantly decreased the FSH level in the blood of rats compared to SG ($P<0.05$). Long-term PRP application, by comparison with the SG, significantly decreased the FSH level (1 (0.07) ng/ml) in the LT-PRP ($P<0.05$).

The immunohistochemical results for ovarian and uterine tissues in this study are summarized in Table 2. When the uterus and ovaries were examined in terms of the presence of Ki-67 and VEGF, a statistically significant increase was observed in the ST-PRP and LT-PRP groups ($P<0.05$).

The histopathological scoring results of uterine tissues were compared between the 3 groups and are summarized in Table 3. After PRP application, while the amounts of fibrosis and inflammation decrease in the uterine tissue, vascular proliferation increases. Compared to the SG group, this effect was statistically significant in the ST-PRP group ($P<0.05$), but not statistically significant in the LT-PRP group ($P>0.05$).

Table 1: The blood hormone results of rats for the three groups

	SG (n:7)	ST-PRP (n:7)	LT-PRP (n:7)	P1	P2	P3
FSH	1.4(0.31)	0.85(0.11)	1(0.07)	0.001	0.005	0.335
AMH	1(0.14)	1.5(0.33)	1(0.11)	0.002	0.889	0.005

P1: Comparison of SG and ST-PRP, P2: Comparison of SG and LT-PRP, P3: Comparison of ST-PRP and LT-PRP

Table 2: The immunohistochemical results of ovarian and uterine tissues for three groups

	SG (n:7)	ST-PRP (n:7)	LT-PRP (n:7)	P1	P2	P3
Ki-67 (ovary)	22(1.9)	37.5(2.5)	28.4(2)	<0.001	<0.001	<0.001
VEGF (ovary)	24.7(2.4)	38.8(2.9)	31.5(2.3)	<0.001	<0.001	<0.001
Ki-67 (uterus)	15.8(1)	31.2(1.6)	25.2(3.7)	<0.001	<0.001	<0.001
VEGF (uterus)	25(1.6)	36.7(1.3)	31.1(1.3)	<0.001	<0.001	<0.001

P1: Comparison of SG and ST-PRP, P2: Comparison of SG and LT-PRP, P3: Comparison of ST-PRP and LT-PRP

Table 3: The histopathological scoring results of uterine tissues for the three groups

Uterus	SG (n:7)	ST-PRP (n:7)	LT-PRP (n:7)	P1	P2	P3
Fibrosis	1.29(0.48)	0.57(0.53)	0.71(0.48)	0.041	0.114	0.858
Inflammation	1.29(0.48)	0.57(0.53)	0.86(0.69)	0.041	0.367	0.631
Vascular proliferation	1.00(0.00)	1.57(0.53)	1.29(0.48)	0.049	0.424	0.424

P1: Comparison of SG and ST-PRP, P2: Comparison of SG and LT-PRP, P3: Comparison of ST-PRP and LT-PRP

The uterine sections of the study groups stained with HE are shown in Figure 1, and the MT-stained sections are shown in Figure 2. The Ki-67 and VEGF stained sections of the uterine and ovarian tissues of the study groups are shown in figures 3, 4, 5 and 6.

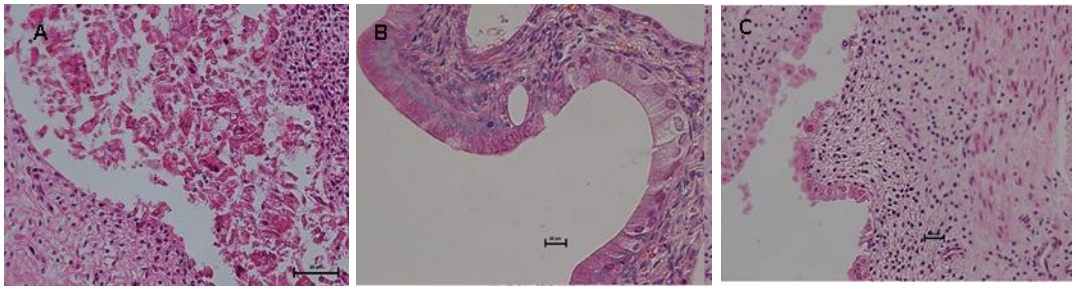

Figure 1: Hematoxylin-eosin (HE) sections from a single rat uterus of each group (×200). (A (SG): The epithelial layer is irregular, the endometrium epithelium has been shed in most areas. B (ST-PRP): The epithelial layer appears smooth and it is noteworthy that it is more regular than both the LT-PRP group and the SG group. C (LT-PRP): Although the epithelial layer is irregular in some areas, the endometrial epithelial cells in the area facing the lumen show a more regular structure compared to the SG group.)

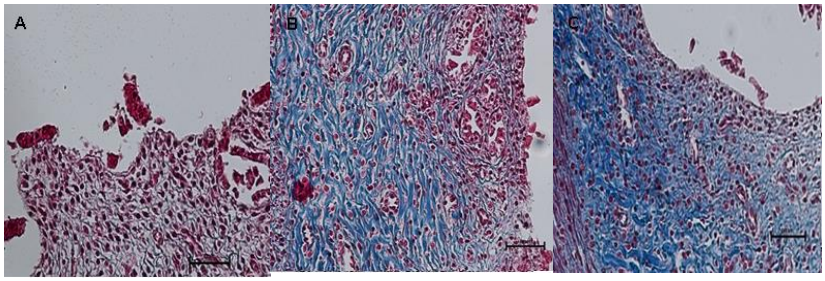

Figure 2: Masson-Trichrome (MT) sections from a single rat uterus of each group (×200). (A (SG): The epithelial layer is irregular, but the fibrosis and collagen fiber areas are observed to be more regular. B (ST-PRP): It seems that the epithelial layer is regular in some areas, and the fibrosis and collagen fiber areas are more regular and dense than the SG and LT-PRP groups. C (LT-PRP): Although the epithelial layer is only a small amount, the fibrosis and collagen fiber areas were extensively evaluated.)

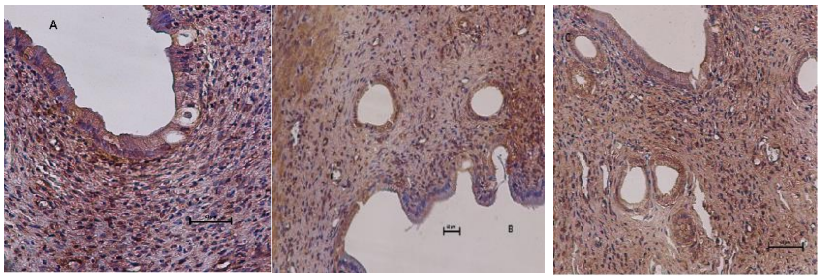

Figure 3: Indication of the activity of Ki-67 expression on the uterus using the IHC staining method for each group (×200). (A (SG), B (ST-PRP), C (LT-PRP): The SG group is less intensely stained than the PRP-treated groups. The expression density of Ki-67 in the ST-PRP group is higher than in the LT-PRP group.)

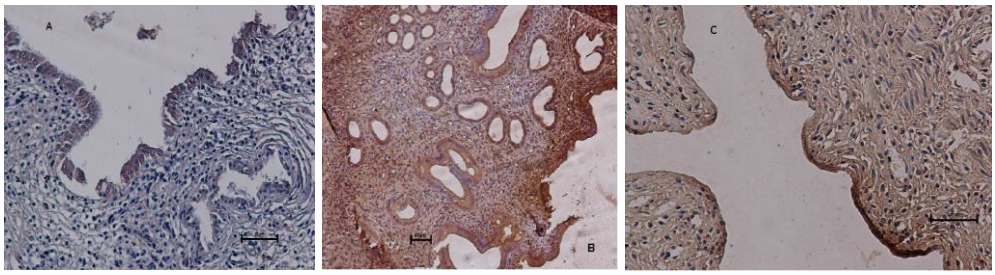

Figure 4: Indication of the activity of VEGF expression on the uterus using the IHC staining method for each group (×200). (A (SG), B (ST-PRP), C (LT-PRP): The SG group is less intensely stained than the PRP-treated groups. VEGF expression intensity in the ST-PRP group is higher than in the LT-PRP group.)

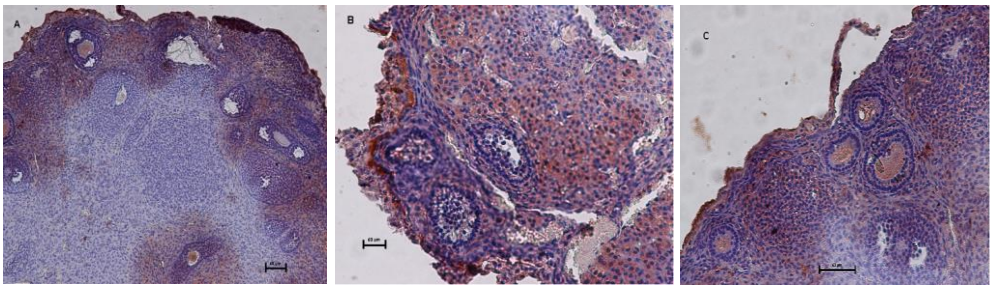

Figure 5: Indication of the activity of Ki-67 expression on the ovaries using the IHC staining method for each group (×200). (A (SG), B (ST-PRP), C (LT-PRP): Compared to the PRP-treated groups, regions close to the germinal epithelium were stained in the SG group. In the ST-PRP and LT-PRP groups, Ki-67 expression was intensely observed over the entire ovarian tissue.)

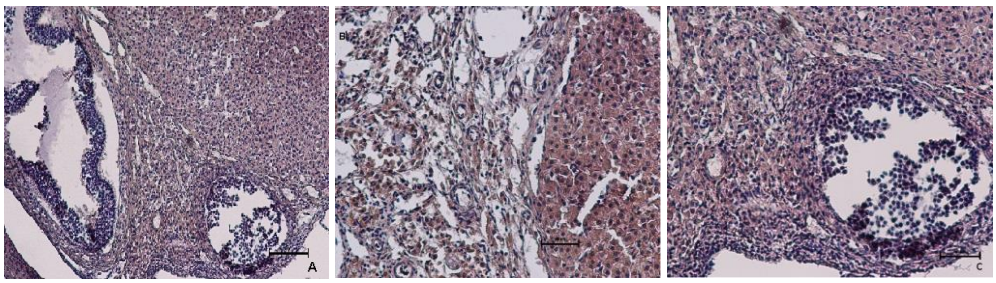

Figure 6: Indication of the activity of VEGF expression on the ovaries using the IHC staining method for each group (×200). (A (SG), B (ST-PRP), C (LT-PRP): The SG group was less intensely stained than the PRP-treated groups. In the ST-PRP and LT-PRP groups, VEGF expression was observed to be intense over the entire ovarian tissue.)

Discussion

PRP is an autologous serum that contains various growth factors and cytokines such as FGF, PDGF, VEGF, TGF-β, IGF-1, IGF-2, CTGF and IL-8 [19]. These molecules are involved in tissue proliferation and regeneration. Some researchers have shown that PRP application has a positive effect on the growth and development of follicles in the ovaries [8]. When the literature was examined, it was seen that PRP applied before intrauterine insemination has a positive effect on the increase of endometrial thickness [20].

When the studies in the field of gynecology were examined, it was seen that PRP application is performed alongside infertility treatments and for a brief time. There is an uncertainty about the long-term effects of this application, which is considered effective and beneficial in the short term in infertility treatments [10,20]. Because of these reasons, this study planned to show – for the first time – the short-term and long-term effects of PRP treatment on uterine and ovarian tissue. The intention was also to try and clarify what caused changes to the parameters like AMH and FSH, which provide information about the reproductive capacity of women.

Sfakianoudis et al. [10] showed that the FSH value decreased, the AMH value increased, and even pregnancy could be achieved in patients with premature menopause following PRP treatment. In this current study, when the SG group and ST-PRP group were compared, it was found that FSH decreased and AMH increased. In addition, when the SG group and LT-PRP group were compared, the decrease observed in FSH was statistically significant. However, there was no difference between the AMH amounts between the two groups. AMH is a marker that provides information about the reproductive capacity in women, regardless of the menstrual cycle. Therefore, by looking at the blood results of the study groups, it can be said that the positive effect of PRP gradually decreased in the long term.

VEGF is the main molecule that plays a role in angiogenesis and vasculogenesis [21]. Ki-67 is known as the main proliferation marker [22]. When Table 2 is examined, it is seen that Ki-67 and VEGF staining increased in both uterine and ovarian tissue in the PRP-treated groups. As a result of the growth factors that PRP contains, tissue proliferation and angiogenesis increased. When the results of the LT-PRP group were examined, the changes in VEGF and Ki-67 expression at the tissue level continued for a long time, contrary to the blood results.

When the histopathological results of the SG group and ST-PRP group were compared for the uterus, it was seen that the amount of inflammation and fibrosis decreased, but vascular proliferation increased. This positive effect of PRP at a tissue level was not seen in the LT-PRP group.

PRP applications are mostly used to increase the success of assisted reproductive techniques in the field of infertility. In order to increase endometrial thickness and pregnancy rates, PRP is applied in the same cycle in which the assisted reproductive technique is applied. The results of this current study support the literature showing that the positive effects of PRP decrease as time goes on [19,23,24].

When Figure 2 is examined, the amount of collagen fiber and fibrosis increased in the stroma of the uterus for PRP-treated groups. It is noteworthy that especially in the LT-PRP group, the amount of collagen fiber increased and became more concentrated. It is thought that this is related to the CTGF which is contained in the PRP. Uncontrolled increases in the amount of connective tissue in the organs can disrupt the microenvironment of the tissues. Uncontrolled increases in connective tissue can lead to decreased flexibility of organs and thus to decreased function of the organs [25]. The increase in the amount of connective tissue in the organs due to CTGF may be the limiting factor in repeated PRP applications.

In the literature, intra-abdominal PRP application is reported to decrease adhesion formation and accelerate wound healing [4]. Spartalis et al. [26] reported that intra-abdominal PRP application would be inconvenient in patient groups undergoing operations for malignancies. It is estimated that growth factors in PRP can increase malignant cell formation and occult metastases.

Limitations

This current study featured some limitations. Firstly, prior to this study, there has been no data about the long-term effects of PRP treatments for uterine and ovarian tissue. Secondly, as the aim of the study was to investigate if PRP had beneficial effects on the uterus and ovaries, only a single and average dose of 1 ml/kg was examined. It would be better to compare different doses of PRP to find the mean effective dose. Thirdly, the study did not examine the follicle counts via histopathology in the ovaries. Counting primordial, developing and atretic follicles could enable us to show the effects of PRP on ovarian tissue in more detail. Fourthly, the results of experimental studies on animals should not be extrapolated to humans. We think that more accurate information will be possible in the future should more studies on this subject be carried out.

Conclusion

As a result of our study, positive effects of PRP application on the ovaries and endometrium are observed on histological and hormonal levels. Since we applied a single dose of PRP in our study, we observed that the positive effects of PRP were more effective in a short time after the application and that the effects of PRP application decreased in the long term. If PRP application is to be preferred as a treatment, we think that applying a cure at regular intervals instead of a single dose application may have longer and permanent effects in terms of treatment efficiency. This idea should be supported by further research.

References

1. Garcia-Velasco JA, Acevedo B, Alvarez C, Monica A, Jose B, Juan F, et al. Strategies to Manage Refractory Endometrium: State of the Art in 2016. Reprod Biomed Online. 2016;32(5):474-89.
2. Sills ES, Wood SH. Autologous Activated Platelet-Rich Plasma Injection Into Adult Human Ovary Tissue: Molecular Mechanism, Analysis, and Discussion of Reproductive Response. Biosci Rep. 2019;39(6):BSR20190805.
3. Lubkowska A, Dolegowska B, Banfi G. Growth Factor Content in PRP and Their Applicability in Medicine. J Biol Regul Homeost Agents. 2012;26(2 Suppl 1):3S-22S.
4. Kaya F, Kismet K, Ozer H, Soylu VG, Duymus ME, Akgun YA, et al. Can Platelet-Rich Plasma Be Used Safely in Intra-Abdominal Operations? Bratisl Lek Listy. 2016;117(9):525-29.
5. Knezevic NN, Candido KD, Desai R, Kaye AD. Is Platelet-Rich Plasma a Future Therapy in Pain Management? Med Clin North Am. 2016;100(1):199-217.
6. Magalon J, Bausset O, Serratrice N, Laurent G, Houssein A, Julie V, et al. Characterization and Comparison of 5 Platelet-Rich Plasma Preparations in a Single-Donor Model. Arthroscopy. 2014;30(5):629-38.

7. Bakacak M, Bostanci MS, İnanc F, Yaylali A, Serin S, Attar R, et al. Protective Effect of Platelet Rich Plasma on Experimental Ischemia/Reperfusion Injury in Rat Ovary. Gynecol Obstet Invest. 2016;81(3):225-31.
8. Hosseini L, Shirazi A, Naderi MM, Shams-Esfandabadi N, Borjian Boroujeni S, Sarvari A, et al. Platelet-rich Plasma Promotes the Development of Isolated Human Primordial and Primary Follicles to the Preantral Stage. Reprod Biomed Online. 2017;35(4):343-50.
9. Sfakianoudis K, Simopoulou M, Nitsos N, Rapani A, Pantou A, Vaxevanoglou T, et al. A Case Series on Platelet-Rich Plasma Revolutionary Management of Poor Responder Patients. Gynecol Obstet Invest. 2019;84(1):99-106.
10. Sfakianoudis K, Simopoulou M, Nitsos N, Rapani A, Pappas A, Pantou A, et al. Autologous Platelet-Rich Plasma Treatment Enables Pregnancy for a Woman in Premature Menopause. J Clin Med. 2018;8(1):1.
11. Nagata MJ, Messora MR, Furlaneto FA, Fucinia SE, Boscoa AF, Garciaa VG, et al. Effectiveness of Two Methods for Preparation of Autologous Platelet-Rich Plasma: An Experimental Study in Rabbits. Eur J Dent. 2010;4(4):395-402.
12. Jang HY, Myoung SM, Choe JM, Kim T, Cheon YP, Kim YM, et al. Effects of Autologous Platelet-Rich Plasma on Regeneration of Damaged Endometrium in Female Rats. Yonsei Med J. 2017;58(6):1195-203.
13. Zhang S, Li P, Yuan Z, Tan J. Platelet-rich Plasma Improves Therapeutic Effects of Menstrual Blood-Derived Stromal Cells in Rat Model of Intrauterine Adhesion. Stem Cell Res Ther. 2019;10(1):61.
14. Irkorucu O, Ferahköşe Z, Memiş L, Ekinci O, Akin M. Reduction of Postsurgical Adhesions in a Rat Model: A Comparative Study. Clinics (Sao Paulo). 2009;64(2):143-8.
15. Yokoyama Y, Takahashi Y, Morishita S, Hashimoto M, Niwa K, Tamaya T. Telomerase Activity in the Human Endometrium Throughout the Menstrual Cycle. Mol Hum Reprod. 1998;4(2):173-7.
16. McCampbell AS, Walker CL, Broaddus RR, Cook JD, Davies PJ. Developmental Reprogramming of IGF Signaling and Susceptibility to Endometrial Hyperplasia in the Rat. Lab Invest. 2008;88(6):615-26.
17. Li X, Sun H, Lin N, Hou X, Wang J, Zhou B, et al. Regeneration of Uterine Horns in Rats by Collagen Scaffolds Loaded With Collagen-Binding Human Basic Fibroblast Growth Factor. Biomaterials. 2011;32(32):8172-81.
18. Ribeiro AA, Martin I, Derussi AA, Ferioli RB, Laufer-Amorim R, Lopes MD. Histological Characterization and Immunohistochemical Localization of ki-67 and VEGF Factors in Bitches' Corpus Luteum at Cyclic and Early and Late Gestational Diestrous. Reprod Domest Anim. 2012;47 Suppl 6:61-5.
19. Nazari L, Salehpour S, Hoseini S, Zadehmodarres S, Ajori L. Effects of autologous platelet-rich plasma on implantation and pregnancy in repeated implantation failure: A pilot study. Int J Reprod Biomed (Yazd). 2016;14(10):625-8.
20. Sipahi M. Effects of Autologous Platelet-Rich Plasma on Endometrium Thickness and Pregnancy Rates During Intrauterine Insemination. Middle Black Sea Journal of Health Science. 2019; 5(2):63-6.
21. Apte RS, Chen DS, Ferrara N. VEGF in Signaling and Disease: Beyond Discovery and Development. Cell. 2019;176(6):1248-64.
22. Sun X, Kaufman PD. Ki-67: more than a proliferation marker. Chromosoma. 2018;127(2):175-186.
23. Chang Y, Li J, Chen Y, Wei L, Yang X, Shi Y, et al. Autologous platelet-rich plasma promotes endometrial growth and improves pregnancy outcome during in vitro fertilization. Int J Clin Exp Med. 2015;8(1):1286-90.
24. Sfakianoudis K, Simopoulou M, Nitsos N, Lazaros L, Rapani A, Pantou A, et al. Successful Implantation and Live Birth Following Autologous Platelet-rich Plasma Treatment for a Patient with Recurrent Implantation Failure and Chronic Endometritis. In Vivo. 2019;33(2):515-21.
25. Wu CK, Wang YC, Lee JK, Chang SN, Su MY, Yeh HM, et al. Connective tissue growth factor and cardiac diastolic dysfunction: human data from the Taiwan diastolic heart failure registry and molecular basis by cellular and animal models. Eur J Heart Fail. 2014;16(2):163-72.
26. Spartalis E, Damaskos C, Athanasiou A. Safety of platelet-rich plasma application in abdominal operations. Bratisl Lek Listy. 2017;118(7):437.

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