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Research Article

Intratesticular Platelet-Rich Plasma (PRP) Application to Improve Testicular Development, Hemodynamic and Testosterone in Male Lambs

Erkek Kuzularda Testiküler Gelişimi, Hemodinamiği ve Testosteron Düzeyini İyileştirmek Amacıyla İntratestiküler Trombositten Zengin Plazma (PRP) Uygulaması

Aslıhan ÇAKIR CİHANGİROĞLU[1,✉], Gaffari TÜRK[2], İbrahim Halil GÜNGÖR[2], Tutku Can ACISU[2], Nida BADILLI[2], Şuheda YÜKSEL[2], Fatma FIRAT DANIŞ[2], Şeyma ÖZER KAYA[2], Seyfettin GÜR[2], Mustafa SÖNMEZ[2]

[1] Department of Reproduction and Artificial Insemination, Faculty of Veterinary Medicine, Siirt University, Siirt, Türkiye
[2] Department of Reproduction and Artificial Insemination, Faculty of Veterinary Medicine, Fırat University, Elazig, Türkiye

Corresponding Author:
Aslıhan ÇAKIR CİHANGİROĞLU
✉aslihan.cakir@siirt.edu.tr

ORCID:
AÇC: 0000-0003-3365-8960
GT: 0000-0001-7417-1038
İHG: 0000-0002-5250-1478
TCA: 0000-0002-0882-937X
NB: 0000-0002-1007-9723
ŞY: 0009-0004-0895-3014
FFD: 0000-0002-0165-7958
ŞÖK: 0000-0002-9970-9364
SG: 0000-0003-0096-2501
MS: 0000-0003-0281-7228

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Abstract

The purpose of this study was to evaluate the effect of periodic intratesticular PRP administration on testicular development in male lambs by examining the changes in testosterone levels, testicular morphometry, and supratesticular artery hemodynamic. The control group lambs (n=16) received an intratesticular injection of saline solution, while the experimental group lambs (n=16) were administered autologous PRP. Injections were administered monthly for a period of 10 months. Following the initial PRP injection in June, testosterone levels, testicular morphometric measurements, and spermatic cord Doppler ultrasonography measurements were assessed on a monthly basis for a period of 10 months (July to April). Platelet counts in whole blood and PRP, and growth factor levels in PRP were also analysed. Testicular blood flow in October, scrotal thickness in December, and testicular length in April were significantly higher in the PRP group compared to the control group ($p<0.05$). Furthermore, the resistance index value among Doppler ultrasonography parameters was observed to be lower in the PRP group compared to the control group ($p<0.05$). Significant inter-month differences were observed in platelet counts in whole blood ($p<0.001$) and PRP ($p<0.001$), as well as in FGF1 levels in PRP ($p<0.05$). It is hypothesized that the administration of PRP to lambs at an early age may exert a slight beneficial influence on testicular development, attributable to the presence of certain growth factors within the PRP.

Key words: Lamb, Platelet-rich plasma, Testosterone, Testicular size, Testicular hemodynamic

Öz

Bu çalışmanın amacı, erkek kuzularda periyodik intratestiküler PRP uygulamasının testiküler gelişim üzerine etkisini; testosteron düzeyleri, testiküler morfometri ve supratestiküler arter hemodinamiğindeki değişiklikleri inceleyerek değerlendirmektir. Kontrol grubundaki kuzulara (n=16) intratestiküler serum fizyolojik enjeksiyonu uygulanırken, deney grubundaki kuzulara (n=16) otolog PRP uygulanmıştır. Enjeksiyonlar 10 ay boyunca aylık olarak gerçekleştirilmiştir. Haziran ayında yapılan ilk PRP uygulamasını takiben, testosteron düzeyleri, testiküler morfometrik ölçümler ve spermatik kordon Doppler ultrasonografi ölçümleri 10 ay süreyle (Temmuz–Nisan) aylık olarak değerlendirilmiştir. Ayrıca tam kanda ve PRP'de trombosit sayıları ile PRP'deki büyüme faktörü düzeyleri analiz edilmiştir. PRP grubunda, Ekim ayında testiküler kan akımı, Aralık ayında skrotal kalınlık ve Nisan ayında testiküler uzunluk kontrol grubuna kıyasla anlamlı derecede daha yüksek bulunmuştur ($p<0,05$). Ayrıca Doppler ultrasonografi parametrelerinden direnç indeksi değerinin PRP grubunda kontrol grubuna göre daha düşük olduğu belirlenmiştir ($p<0,05$). Tam kandaki trombosit sayılarında ($p<0,001$), PRP'deki trombosit sayılarında ($p<0,001$) ve PRP'deki FGF1 düzeylerinde ($p<0,05$) aylar arasında anlamlı farklılıklar gözlenmiştir. Erken yaşta kuzulara PRP uygulanmasının, PRP içeriğinde bulunan bazı büyüme faktörlerine bağlı olarak testiküler gelişim üzerinde hafif düzeyde olumlu bir etki gösterebileceği düşünülmektedir.

Anahtar kelimeler: Kuzu, Platelet-rich plasma, Testosteron, Testis büyüklüğü, Testiküler hemodinami.

Introduction

In domestic animals, the testes, which are located outside the body and suspended by the spermatic cord, are a pair of organs that are responsible for exocrine and endocrine activities. The testes form in proximity to the kidneys during the embryonic period and descend into the scrotal sac in mid-pregnancy in domestic animals. Consequently, in new-born lambs, the testes should be located within the sac. The size of the testes varies depending on the species, nutrition, mating season, and age (Bearden et al., 2003). During the foetal period, the testes synthesize the testosterone hormone, which is responsible for the development of the Wolffian duct. This occurs through the synthesis of human chorionic gonadotrophin (hCG) from the placenta and interstitial cell stimulating hormone (ICSH) from the adenohypophysis. The majority of testosterone secreted by Leydig cells is transported by the blood to target cells, while the remainder is converted into active dihydrotestosterone. Testosterone is the most significant endocrine factor influencing male fertility and plays a pivotal role in sexual differentiation during the foetal period and in the development of sexual behaviour, spermatozoon production, and quality during the pubertal period (Fernandez-Abella et al., 1999; Gromoll et al., 2000; Khalifa et al., 2013; Saeed and Zaid, 2019).

Postnatal testicular development is characterized by the proliferation and differentiation of gonocytes, which subsequently undergo spermatogenesis. In the context of prepubertal testicular development, these cells undergo a series of distinct developmental stages (Oatley et al., 2004; Nazari-Zenouz et al., 2016). In rams, gonocytes differentiate into spermatogonial stem cells during the postnatal period, with early spermatogenesis commencing at approximately 9-12 weeks (Herrera-Alarcón et al., 2007). The secretion of the testosterone hormone, which plays a role in the mechanism of testicular development and spermatogenesis, fluctuates and correlates with the weight and age of the testes during the prepubertal period in rams (Saeed and Zaid, 2019). Elmaz et al. (2007), who investigated the changes in testicular dimensions related to postnatal testicular development in lambs, found that scrotal circumference, testicular length, and testicular volume in two-month-old Kivircik lambs were 9.52 cm, 2.83 cm, and 10.8 cm^3, respectively. At 100 days of age, these values reached 10.88 cm, 3.39 cm, and 17.3 cm^3, respectively, while at one year of age, they reached 29.68 cm, 11.56 cm, and 411.9 cm^3, respectively.

Adequate blood flow is a prerequisite for the maintenance of the physiological functions and homeostasis of the testes. The hemodynamic of the blood supply to the testes is determined by colour Doppler scans (Velasco and Ruiz, 2020). In addition to its use in the evaluation of testicular hemodynamic, colour Doppler ultrasound is a valuable tool for the assessment of various functions, including testicular morphology, spermatogenesis, and steroidogenesis. It is also a crucial technique for the diagnosis of infertility cases. The most common hemodynamic parameters measured by colour Doppler are peak systolic velocity (PSV), end-diastolic velocity (EDV), resistance index (RI), and pulsatility index (PI) (Pozor and McDonnell, 2004; Pozor, 2007; Pinggera et al., 2008; Hedia et al., 2019; Hedia and El-Belely, 2021; Kaya et al., 2025).

In addition to the endocrine mechanism in the hypothalamic-pituitary-gonadal axis, the paracrine/autocrine system plays a role in maintaining male and female gonadogenesis and functions. Growth factors are of particular importance within the paracrine/autocrine system, influencing testicular organogenesis, spermatogenesis, and the steroidogenesis mechanism in males by stimulating cell growth, proliferation, and differentiation (Basciani et al., 2010). In consideration of the product with the highest concentration of growth factors, PRP is the most prominent candidate. PRP is defined as blood plasma containing a high concentration of platelets, which is on average three times higher than the physiologic limits. This concentration is achieved through the centrifugation of whole blood. PRP contains a multitude of growth factors, predominantly platelet-derived growth factor (PDGF) and its isomers (PDGF$\alpha\alpha$, PDGFββ, and PDGFαβ), vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), epithelial growth factor (EGF), transforming growth factor β (TGFβ) and its isomers (TGFβ1 and TGFβ2), and insulin-like growth factor (IGF). In addition to their roles in growth, proliferation, and differentiation, growth factors also perform a number of other functions, including chemotaxis, anti-inflammatory activity, anti-apoptotic effects, and the promotion of regeneration (Matz et al., 2018; Epifanova et al., 2020).

PRP has been employed in a number of medical fields, including cardiology (Patel et al., 2016), ophthalmology (Anitua et al., 2016), plastic surgery (Taschieri et al., 2017), neurology (Teymur et al., 2017), orthopedic surgery and sports medicine (Foster et al., 2009), cosmetology (Cameli et al., 2017), androgenetic alopecia (Donnelly et al., 2024), dentistry (de Obarrio et al., 2000), bone defect treatment (Camargo et al., 2002), diabetes and its complications (Ruiz-Muñoz et al., 2024). Recently, there has been a notable increase in the number of studies examining the potential applications of PRP in male (Haidar, 2024) and female (Vali et al., 2023) reproductive health. Nevertheless, there is a paucity of data regarding the impact of intratesticular PRP administration on testicular development in lambs. Therefore, this study was conducted to evaluate the effect of periodic intratesticular PRP administration on testicular development in male lambs by examining the changes in testosterone levels, testicular size, and supratesticular artery hemodynamic. In addition, platelet counts in whole blood and PRP, and growth factor levels in PRP were also analysed.

Materials and Methods

Animals and Experimental Design

The experimental phase of this study was carried out with the approval of Fırat University Animal Experiments Local Ethics Committee (Protocol No: 2020/05). The study utilized 32 male Akkaraman lambs, aged two months, free of any systemic and genital diseases. The animals were housed in paddocks within the Hospitalization Unit of Fırat University Animal Hospital, with a density of seven to eight animals per paddock. The lambs were fed a diet of lamb growth feed, barley, and hay until they reached six months of age. Thereafter, they were fed a diet of lamb fattening feed, barley, and hay. The animals had access to drinking water at all times. The study commenced when the animals were two months old and ended when they reached 12 months of age. Given that the majority of testicular development in small ruminants is typically complete by one year of age, the study was conducted over a 10-month period. The animals were divided into two groups as experimental and control groups with 16 lambs in each group. The first group (PRP) received their initial PRP injection at two months of age, with subsequent injections administered monthly until the 12-month mark. This resulted in a total of 10 injections between June and March. The second group (Control) received 0.9% saline (placebo) injections instead of PRP, thereby creating a stress response comparable to that of the PRP injections. Starting one month after the first PRP injection, samples and measurements were collected and analysed monthly for 10 months (from July to April). After the first PRP injection, animals in both groups were castrated once a month and removed from the study. Oxidative stress, histopathological, immunohistochemical, and molecular analyses were performed on the testes. The analyses obtained from the testes are not included in this article.

Blood Collection and Preparation of PRP

In the study, autologous PRP was used to prevent antigenic effect. PRP was prepared under aseptic conditions to prevent microbial contamination. Blood samples were collected monthly during the study period. For the purpose of blood sampling, the neck of the animal was meticulously cleansed with an antiseptic solution and cotton wool. To obtain the appropriate PRP dose and to count platelet in PRP, 20 mL blood was collected from each animal in the experimental group using a sterile vacutainer and sterile tubes containing anticoagulant sodium citrate. In addition, sufficient amount of blood was taken from the animals in each group to determine the plasma total testosterone level and platelet count in the PRP group and the plasma testosterone level in the control group. For this, blood samples were centrifuged at 1500 xg for 5 minutes and the plasma samples obtained were stored at -20 °C until being analysed.

Pure, leukocyte-poor PRP was obtained at room temperature by modifying the double centrifugation method reported by Mariano et al. (2010). The 20-mL blood samples from the PRP group underwent a 20-min centrifugation at 160 xg. The initial centrifugation procedure resulted in the separation of the blood into three distinct layers: erythrocytes at the bottom, buffy coat in the middle, and yellowish plasma at the top. The remaining upper 1/3 of the plasma and the middle layer, which is rich in leukocytes and platelets and is referred to as the buffy coat, were transferred to different sterile tubes that did not contain anticoagulants and were subjected to a second centrifugation process at 400 xg for 15 minutes. Following second centrifugation, the upper 2/3 of the sample, comprising platelet-poor plasma, was discarded. The lower 1/3, containing an average of 4-5 mL, was deemed to represent PRP. As a consequence of the leukocyte-poor nature of the PRP obtained by this method (Dhurat and Sukesh, 2014) the potential for pain or inflammation at the injection site was also eliminated. From the PRP samples obtained, 0.5 mL was separated and the platelet count was determined. Furthermore, 1 mL was separated and stored at -80 °C for subsequent growth factor analysis. Despite reports indicating that the amount of growth factors secreted from PRPs stored at room temperature for up to six hours remains unchanged (Hauschild et al., 2016), as well as platelet aggregation (Ho and Chan, 1995), PRPs were injected into animals within a maximum of 20 min to prevent pH changes due to lactic acid accumulation and to avoid changes in the level of growth factors in PRP. There is a divergence of opinion among researchers regarding the activation of PRP prior to its application. Some researchers posit that PRP should be activated with thrombin or calcium-chloride ($CaCl_2$) to ensure the secretion of growth factors from platelets. However, others argue that activation is not necessary because platelets degranulate in the external environment or are activated when they encounter type I collagen in the injected tissue (Yılmaz and Kesikburun, 2013; Dhurat and Sukesh, 2014). The activation of PRP results in the formation of a gel, which may preclude its use in injection (Mishra et al., 2009). Accordingly, the present study employed one PRP sample for comparative analysis of growth factor levels in $CaCl_2$-activated and non-activated PRPs. The results of the analysis demonstrated that no significant differences were observed (Table 1). Therefore, non-activated pure PRP was injected in the study.

Table 1. Growth factor levels measured by ELISA in one PRP sample with and without $CaCl_2$

	VEGF ($ng{\cdot}L^{-1}$)	**FGF1 ($ng{\cdot}L^{-1}$)**	**IGF1 ($ng{\cdot}mL^{-1}$)**	**TGFβ ($ng{\cdot}L^{-1}$)**	**PDGF ($ng{\cdot}mL^{-1}$)**
$CaCl_2$ (+) PRP	182.00	75.63	82.93	122.40	0.76
$CaCl_2$ (-) PRP	204.40	80.57	120.20	120.20	1.27

Determination of Platelet Count

An automated haematology device (BC-5000Vet, Mindray, Guangdong, China) was used to determine platelet count in the PRP to be injected into the animals and to compare with the levels in whole blood. The amount of PRP administered intratesticularly to the experimental group was adjusted according to the platelet count.

Analysis of Growth Factors in PRP

Following the thawing of the PRP samples stored at -80 °C, growth factor analyses were conducted via enzyme-linked immunosorbent assay (ELISA) method using specific sheep kits, in accordance with the instructions provided by the kit manufacturer (Sunred Biological Technology, Co., Ltd., Baoshan, China). Among the growth factors present in PRP, the levels of VEGF (Sheep VEGF ELISA kit, Catalog No: 201-07-2673), FGF1 (Sheep FGF1 ELISA kit, Catalog No: 201-07-0030), IGF1 (Sheep IGF1 ELISA kit, Catalog No: 201-07-0005), TGFβ (Sheep TGFβ ELISA kit, Catalog No: 201-07-3014) and PDGF (Sheep PDGF ELISA kit, Catalog No: 201-07-3618) were determined by ELISA reader (Multiskan FC, ThermoFisher Scientific, Waltham, Massachusetts, USA).

Intratesticular PRP Application

There are four different classifications regarding the number of platelets in PRP administered in one application: a) very high platelet concentration (>5 billion), b) high platelet concentration (3-5 billion), c) moderate platelet concentration (1-3 billion), d) low platelet concentration (<1 billion) (Alves and Grimalt, 2018). In general, it is suggested that the amount of PRP containing 2-3 billion platelets in one injection is the ideal application dose (Le et al., 2018). In light of this information, in this study, a moderate amount (approximately 1-3 mL) of sterile PRP (2-3 billion platelets) was injected monthly into the central part of each testicle after scrotal shaving and antisepsis with 10% povidone-iodine solution (Poviiodeks 1000 mL, Kim-Pa İlaç, İstanbul, Türkiye). The control group was injected with the same amount of 0.9% saline (Figure 1).

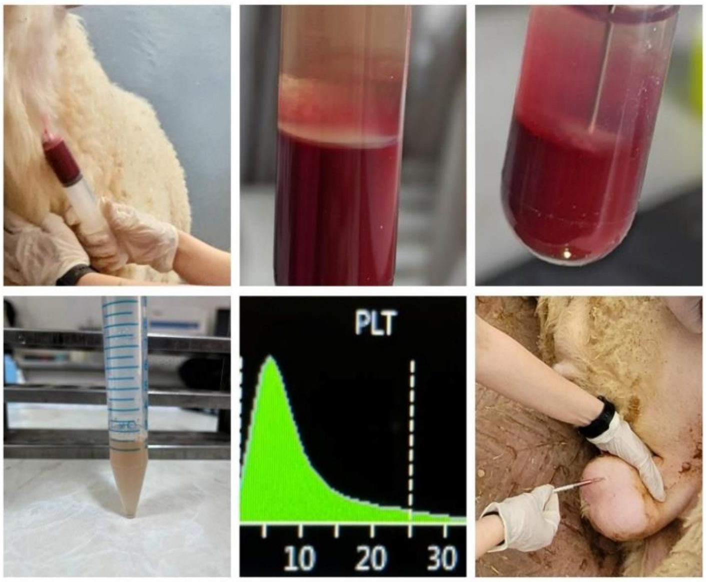

Figure 1. Preparation and intratesticular application of PRP.

Plasma Total Testosterone Measurement

After thawing the plasma samples stored at -20 °C, the total testosterone level was determined by using a chemiluminescent immunoassay method and specific kit (Siemens ADVIA Centaur Testosterone TSTII, Siemens Healthcare Diagnostics, Erlangen, Germany) on an automated device (Siemens ADVIA Centaur XP, Siemens Healthcare Diagnostics, Erlangen, Germany), and the results were expressed as $ng \cdot dl^{-1}$.

Testicular Morphometric Measurements

Scrotal thickness, and the circumference, length, width and thickness of each testicle over the scrotum, and testicular volume were measured monthly during the study period. Scrotal thickness was measured using a calliper, and scrotal circumference was measured using a tape measure at the widest part of the testicle compressed at the spermatic cord. The length of the testes was measured with the help of a calliper, taking into account the distance from the point where the funiculus spermaticus enters the testis to the underside of the cauda epididymis. The width of the testes was measured with a calliper, taking into account the distance between the centre of the testicular margin and the rafe scroti. Testicular thickness was measured by pinching the centre of each testicle with a calliper and measuring the thickness of each testicle over the scrotum. To measure the volume of the testes, after the cremaster muscle was relaxed by manual massage, a 2 L container was filled with water at 38 °C and both testes were immersed in the water. The volume of overflowing water in this container, including both testes and scrotum, was determined as testicular volume in mL (Aksoy et al., 1994; Gündoğan et al., 2003; Türk et al., 2005) (Figure 2).

Figure 2. Testicular morphometric measurements.

Measurement of Testicular Arterial Hemodynamic

Transcutaneous images of the distal supra-testicular artery were obtained because the curved part of the artery supplying the testis (distal supra-testicular artery) at the junction of the spermatic cord and the testis is the optimal location for Doppler imaging and spectral waveform analysis (Venianaki et al., 2023). A portable colour Doppler ultrasound (Fujifilm-Sonosite-M-Turbo, Tokyo, Japan) and a 7.5 MHz probe were utilized. The scrotum was shaved at the point of contact between the testis and the spermatic cord to prevent the formation of air bubbles, and the probe was coated with an adequate quantity of gel to facilitate imaging (Samir et al., 2018). The angle between the Doppler beam and the long axis of the vessels was adjusted to a value of ≤60°. Among the testicular artery hemodynamic parameters, peak systolic velocity (PSV), end diastolic velocity (EDV), resistive index (RI=PSV-EDV/PSV) and blood flow volume (ml·min^{-1}) values were recorded (Camela et al., 2019; Hedia et al., 2019).

Statistical Analysis

The statistical analysis was conducted using the SPSS 22.0 software (IBM Corp., Armonk, NY, USA). Initially, the data were evaluated for normality. The differences observed between months in terms of platelet counts in PRP and whole blood, and growth factors in PRP were compared using Bonferroni-corrected two-way analysis of variance for repeated measures for normally distributed parameters, and Friedman test followed by Wilcoxon test for non-normally distributed parameters. Similarly, the tests given for platelet count and growth factors analysis were used to determine the differences observed between months within the same group in terms of plasma testosterone levels, and testicular morphometric and hemodynamic measurements. In order to compare the differences between the control and PRP groups in terms of all parameters at each month, student t-test was used for normally distributed parameters and Mann-Whitney-U test was used for non-normally distributed parameters. The data for normally distributed parameters in tables and figures are presented as mean ± standard error (SE) values, while data for non-normally distributed parameters in tables are presented as mean ± SE values and also median values. A P-value of less than 0.05 was considered statistically significant.

Results

Platelet Counts in Whole Blood and PRP

Monthly platelet counts in whole blood and PRP are demonstrated in Figure 3. Significant differences were observed between months in terms of platelet counts in both whole blood ($p<0.001$) and PRP ($p<0.001$) samples. Considering the average platelet counts over 10 months, it was found that the platelet count obtained from the PRP sample was 4.41 times higher than that in whole blood.

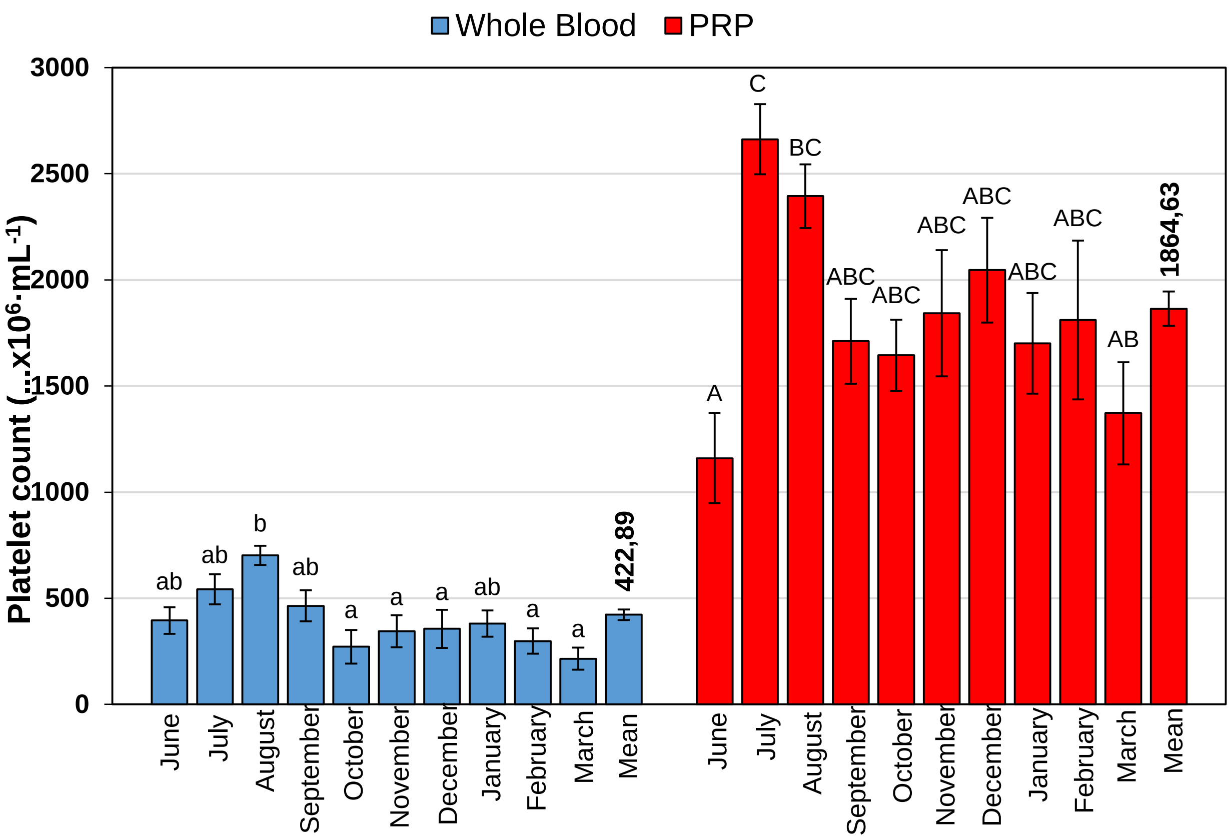

Figure 3. Monthly platelet counts in whole blood and PRP.

The lowercase letters (a, b; $p<0.001$) displayed in the columns indicate the differences in platelet counts obtained from whole blood, while the uppercase letters (A, B, C; $p<0.001$) indicate the differences in platelet counts obtained from PRP according to months.

Growth Factor Levels in PRP

Monthly growth factor levels in PRP samples are presented in Table 2. Differences observed in FGF1 levels between months were statistically significant ($p<0.05$). However, the differences observed in VEGF, IGF1, TGFβ, and PDGF levels between months were not statistically significant. The intratesticularly injected PRP contained high levels of IGF1 (mean: 75.18 ng·mL^{-1} = 75180.00 ng·L^{-1}), which was 359.9 times higher than VEGF, 1094.8 times higher than FGF1, 777.8 times higher than TGFβ, and 53.7 times higher than PDGF.

Table 2. Monthly growth factor levels in PRP samples

Month		**Growth Factors**				
		VEGF (ng·L^{-1})	**FGF1 (ng·L^{-1})**	**IGF1 (ng·mL^{-1})**	**TGFβ (ng·L^{-1})**	**PDGF (ng·mL^{-1})**
June	Mean±SE	187.76±7.02	63.95±3.82^{ab}	81.21±4.52	129.20±10.00	2.39±0.71
	Median	191.50	61.77	83.67	-	1.73
July	Mean±SE	180.32±18.80	54.99±3.54^{ac}	72.09±7.54	98.86±6.57	1.86±0.55
	Median	176.70	54.87	65.11	-	1.26
August	Mean±SE	189.03±5.61	64.40±4.06^{bd}	71.43±3.48	97.48±7.04	1.59±0.21
	Median	191.95	61.52	70.06	-	1.60
September	Mean±SE	202.87±48.20	80.99±10.50^{bde}	81.70±19.13	98.81±12.76	1.70±0.50
	Median	153.70	80.72	62.17	-	0.99
October	Mean±SE	200.38±20.96	70.83±10.22^{bde}	63.39±8.35	86.17±11.81	1.50±0.23
	Median	192.64	75.84	57.68	-	1.46
November	Mean±SE	166.77±24.88	59.08±4.41^{bc}	68.04±5.04	92.24±5.54	1.07±0.11
	Median	157.40	54.51	62.57	-	1.03
December	Mean±SE	196.13±13.76	56.58±3.74^{bc}	62.21±4.51	79.57±8.22	1.03±0.07
	Median	204.30	54.66	59.05	-	0.99
January	Mean±SE	234.68±30.00	71.20±12.26^{bde}	84.69±6.05	93.85±9.36	1.07±0.08
	Median	213.35	63.10	84.21	-	1.08
February	Mean±SE	237.22±29.86	78.61±8.47^{de}	82.49±8.84	98.66±9.53	0.91±0.03
	Median	215.30	84.63	75.85	-	0.89
March	Mean±SE	293.58±35.11	86.06±7.15^{e}	84.50±12.47	91.66±11.90	0.92±0.03
	Median	252.25	90.30	80.97	-	0.92
Mean	Mean±SE	208.87±8.97	68.67±2.56	75.18±2.88	96.65±3.21	1.40±0.12
	Median	198.26	63.95	70.62	-	1.04

Differences among values carrying different lowercase letters (a-e) within the same column are statistically significant ($p<0.05$). Median values are presented for data that do not follow a normal distribution

Plasma Total Testosterone Levels

Table 3 illustrates the plasma total testosterone levels in the control and PRP groups. Accordingly, statistically significant ($p<0.05$) differences in testosterone levels were observed in each group across the months. Nevertheless, no statistically significant differences were observed when the experimental and control groups were compared within the same months. A comparison of the mean testosterone levels over the 10-month period of the control and PRP groups revealed that, although testosterone levels were higher in the PRP-treated experimental group than in the control group, the difference was not statistically significant.

Table 3. Monthly testosterone levels in control and PRP groups

Month	n	Testosterone ($ng \cdot dL^{-1}$) Control		PRP		t-value	p-value
		Mean±SE	Median	Mean±SE	Median		
July	16	130.18±24.05^{a}	-	133.85±23.09^{a}	-	-0.110	0.913
August	15	201.35±39.74^{a}	219.95	167.81±33.61^{ac}	173.17	-	0.541
September	14	191.24±41.93^{acd}	140.63	259.53±61.24^{abc}	232.12	-	0.511
October	13	379.04±68.85^{be}	-	396.56±66.84^{be}	-	-0.183	0.857
November	12	189.62±44.83^{acd}	223.87	210.09±33.69^{abcd}	253.56	-	0.699
December	11	137.15±48.56^{ad}	64.34	286.18±76.94^{abd}	275.90	-	0.105
January	10	196.90±69.75^{abd}	109.68	217.69±68.79^{abd}	120.50	-	0.796
February	9	175.39±42.15^{abe}	-	188.58±43.99^{abe}	-	-0.217	0.832
March	8	376.16±68.52^{ce}	-	402.60±85.15^{ce}	-	-0.242	0.813
April	7	454.18±97.88^{de}	-	477.60±99.03^{de}	-	-0.168	0.870
Mean	115	226.64±18.44	179.43	255.37±19.83	212.91	-	0.276

-Differences between values carrying different lowercase letters (a-e) within the same column are statistically significant ($p<0.05$).

-Median values are presented for data that do not follow a normal distribution, while t-values are presented for data that follow a normal distribution.

Testicular Morphometric Values

The values of thickness and width, length and volume, scrotal circumference length and scrotal thickness parameters, as measured in the testes of animals in the control and PRP groups over the 10-month period, are presented in Tables 4, 5, 6, 7, 8, 9 respectively. When all parameters were considered, the differences observed between months within the same group were statistically significant ($p<0.05$). However, although the numerical differences observed between the control and PRP groups in terms of mean and same-month testicular thickness, width, volume, and scrotal circumference length were not statistically significant, the testicular length values in April and scrotal thickness values in December were significantly higher in the PRP group than in the control group ($p<0.05$).

Table 4. Monthly values of testicular thickness of animals in control and PRP groups

		Testis Thickness (cm, right+left/2)					
		Control		**PRP**			
Month	**n**	**Mean±SE**	**Median**	**Mean±SE**	**Median**	**t-value**	**p-value**
July	16	3.54±0.17^{a}	-	3.43±0.20^{a}	-	0.422	0.676
August	15	3.98±0.15^{a}	-	4.03±0.16^{b}	-	-0.212	0.834
September	14	4.78±0.16^{c}	-	4.97±0.15^{c}	-	-0.885	0.385
October	13	5.38±0.18^{dfgh}	-	5.52±0.15^{de}	-	-0.581	0.567
November	12	5.87±0.15^{ei}	-	5.94±0.15^{de}	-	-0.333	0.742
December	11	5.74±0.15^{deh}	-	5.86±0.14^{de}	-	-0.606	0.552
January	10	5.33±0.08^{f}	-	5.50±0.16^{cde}	-	-0.918	0.372
February	9	5.18±0.05^{gj}	-	5.07±0.19^{c}	-	0.541	0.597
March	8	5.28±0.17^{cdefg}	-	5.46±0.16^{cd}	-	-0.754	0.465
April	7	5.76±0.19^{hij}	5.73	5.86±0.15^{e}	6.01	-	0.818
Mean	115	4.94±0.09	5.11	5.00±0.10	5.21	-	0.467

-Differences between values carrying different lowercase letters (a-j) within the same column are statistically significant ($p<0.05$).

-Median values are presented for data that do not follow a normal distribution, while t-values are presented for data that follow a normal distribution.

Table 5. Monthly values of testicular width of animals in control and PRP groups

		Testis Width (cm, right+left/2)					
		Control		PRP			
Month	n	**Mean±SE**	**Median**	**Mean±SE**	**Median**	**t-value**	**p-value**
July	16	3.60±0.13[a]	-	3.64±0.18[a]	-	-0.172	0.865
August	15	4.48±0.12[b]	-	4.54±0.12[b]	-	-0.376	0.710
September	14	4.77±0.14[b]	-	4.96±0.12[c]	-	-1.017	0.319
October	13	5.25±0.13[ce]	-	5.50±0.14[de]	-	-1.330	0.197
November	12	5.73±0.10[d]	-	5.81±0.12[de]	-	-0.553	0.586
December	11	5.59±0.09[d]	-	5.59±0.08[de]	-	-0.032	0.975
January	10	5.23±0.08[ce]	-	5.36±0.08[cde]	-	-1.177	0.256
February	9	4.78±0.33[bc]	4.99	5.22±0.17[cd]	5.28	-	0.382
March	8	5.46±0.12[de]	-	5.45±0.12[cd]	-	0.067	0.948
April	7	5.81±0.16[f]	-	5.76±0.18[e]	-	0.203	0.843
Mean	115	4.94±0.08	5.10	5.05±0.08	5.19	-	0.335

-Differences between values carrying different lowercase letters (a-f) within the same column are statistically significant ($p<0.05$).

-Median values are presented for data that do not follow a normal distribution, while t-values are presented for data that follow a normal distribution.

Table 6. Monthly values of testicular length of animals in control and PRP groups

		Testis Length (cm, right+left/2)					
		Control		PRP			
Month	**n**	**Mean±SE**	**Median**	**Mean±SE**	**Median**	**t-value**	**p-value**
July	16	5.97±0.32^{a}	-	6.13±0.38^{a}	-	-0.318	0.753
August	15	7.62±0.26^{b}	-	7.76±0.28^{b}	-	-0.357	0.724
September	14	8.84±0.28$^{\text{cgı}}$	-	8.97±0.34^{cf}	-	-0.311	0.759
October	13	9.62±0.28^{dhj}	-	9.77±0.31^{deg}	-	-0.380	0.708
November	12	10.50±0.29^{ef}	-	10.58±0.43^{dg}	-	-0.153	0.880
December	11	10.57±0.33^{e}	-	10.62±0.47^{deg}	-	-0.090	0.929
January	10	9.66±0.25$^{\text{fcdı}}$	-	9.62±0.44^{cdg}	-	-0.065	0.949
February	9	8.94±0.16^{gh}	-	9.01±0.43^{ef}	-	-0.154	0.880
March	8	8.75±0.63^{bcdfg}	9.26	9.81±0.44^{de}	10.09	-	0.610
April	7	9.76±0.24$^{\text{eıj}}$	-	10.87±0.34^{g*}	-	-2.701	**0.022**
Mean	115	8.82±0.17	-	9.03±0.19	-	-0.821	0.413

-*Significant from control ($p<0.05$).

-Differences between values carrying different lowercase letters (a-j) within the same column are statistically significant ($p<0.05$).

-Median values are presented for data that do not follow a normal distribution, while t-values are presented for data that follow a normal distribution.

Table 7. Monthly values of testicular volume of animals in control and PRP groups

		Testis Volume (mL)					
		Control		PRP			
Month	n	Mean±SE	Median	Mean±SE	Median	t-value	p-value
July	16	204.00±15.58[a]	190.00	206.88±23.00[a]	192.50	-	0.770
August	15	288.57±18.63[b]	325.00	291.33±18.92[b]	280.00	-	0.747
September	14	356.15±20.40[c]	330.00	376.43±22.57[c]	335.00	-	0.583
October	13	440.00±23.84[d]	-	451.54±26.24[d]	-	-0.324	0.749
November	12	544.55±35.96[efgh]	-	546.67±48.04[def]	-	-0.035	0.973
December	11	532.00±37.53[efg]	500.00	583.00±44.22[ef]	515.00	-	0.631
January	10	468.89±15.85[de]	480.00	478.89±43.44[de]	480.00	-	0.863
February	9	517.50±14.11[f]	-	523.75±21.29[de]	-	-0.245	0.810
March	8	577.14±13.04[g]	-	588.57±22.72[e]	-	-0.436	0.670
April	7	623.33±15.85[h]	-	655.00±28.14[f]	-	-0.981	0.350
Mean	115	423.43±14.76	450.00	434.19±16.31	440.00	-	0.773

-Differences between values carrying different lowercase letters (a-h) within the same column are statistically significant ($p<0.05$).

-Median values are presented for data that do not follow a normal distribution, while t-values are presented for data that follow a normal distribution.

Table 8. Monthly values of scrotal circumference of animals in control and PRP groups

		Scrotal Circumference (cm)					
		Control		PRP			
Month	**n**	**Mean±SE**	**Median**	**Mean±SE**	**Median**	**t-value**	**p-value**
July	16	18.24±0.74[a]	-	18.28±0.88[a]	-	-0.038	0.970
August	15	22.19±0.59[b]	-	22.31±0.65[b]	-	-0.129	0.898
September	14	24.74±0.71[c]	-	25.19±0.68[c]	-	-0.455	0.653
October	13	27.13±0.73[df]	-	27.21±0.55[df]	-	-0.092	0.928
November	12	28.75±0.47[eg]	-	28.83±0.60[eg]	-	-0.103	0.919
December	11	28.42±0.46[deg]	-	28.81±0.64[de]	-	-0.496	0.626
January	10	27.19±0.37[f]	-	27.92±0.72[cde]	-	-0.906	0.378
February	9	27.06±0.45[def]	-	27.74±0.71[cde]	-	-0.807	0.433
March	8	27.56±0.48[ef]	-	28.27±0.82[de]	-	-0.749	0.468
April	7	29.18±0.45[g]	-	29.90±0.96[fg]	-	-0.677	0.514
Mean	115	25.34±0.40	26.40	25.62±0.42	26.40	-	0.625

-Differences between values carrying different lowercase letters (a-g) within the same column are statistically significant ($p<0.05$).

-Median values are presented for data that do not follow a normal distribution, while t-values are presented for data that follow a normal distribution.

Table 9. Monthly values of scrotal thickness of animals in control and PRP groups

		Scrotal Thickness (cm)					
		Control		PRP			
Month	n	**Mean±SE**	**Median**	**Mean±SE**	**Median**	**t-value**	**p-value**
July	16	0.384±0.014[ac]	-	0.349±0.020[a]	-	1.407	0.170
August	15	0.421±0.015[bde]	-	0.394±0.019[abe]	-	1.102	0.280
September	14	0.391±0.017[ab]	0.400	0.397±0.011[ae]	0.400	-	0.550
October	13	0.411±0.015[cde]	0.400	0.415±0.011[bd]	0.400	-	0.503
November	12	0.431±0.015[de]	0.420	0.446±0.013[cf]	0.435	-	0.487
December	11	0.421±0.011[bcde]	0.400	0.460±0.009[c*]	0.450	-	**0.011**
January	10	0.423±0.017[bcde]	0.400	0.421±0.011[dg]	0.400	-	1.000
February	9	0.394±0.012[bcde]	-	0.406±0.004[def]	-	-0.999	0.335
March	8	0.407±0.013[bcd]	0.400	0.409±0.004[df]	0.400	-	0.805
April	7	0.435±0.017[ae]	0.420	0.437±0.016[cfg]	0.435	-	0.937
Mean	115	0.412±0.006	0.400	0.413±0.009	0.400	-	0.775

-*Significant from control (p<0.05).

-Differences between values carrying different lowercase letters (a-g) within the same column are statistically significant (p<0.05).

-Median values are presented for data that do not follow a normal distribution, while t-values are presented for data that follow a normal distribution.

Testicular Hemodynamic Values

Colour Doppler ultrasound images of the supra-testicular artery are presented in Figure 4. PSV and EDV values measured by Doppler ultrasound in the spermatic cords of animals in the control and PRP groups over the 10-month period are presented in Tables 10 and 11, while the values of RI and blood flow volume parameters are given in Tables 12 and 13. When PSV, EDV, and flow volume were considered, the differences observed between months within the same group were statistically significant ($p<0.05$). The differences observed between months in terms of RI values in the control group were also significant ($p<0.05$). The mean RI value of the PRP group was found to be significantly lower than that of the control group ($p<0.05$), while the flow volume in October was found to be significantly higher than that of the control group ($p<0.05$).

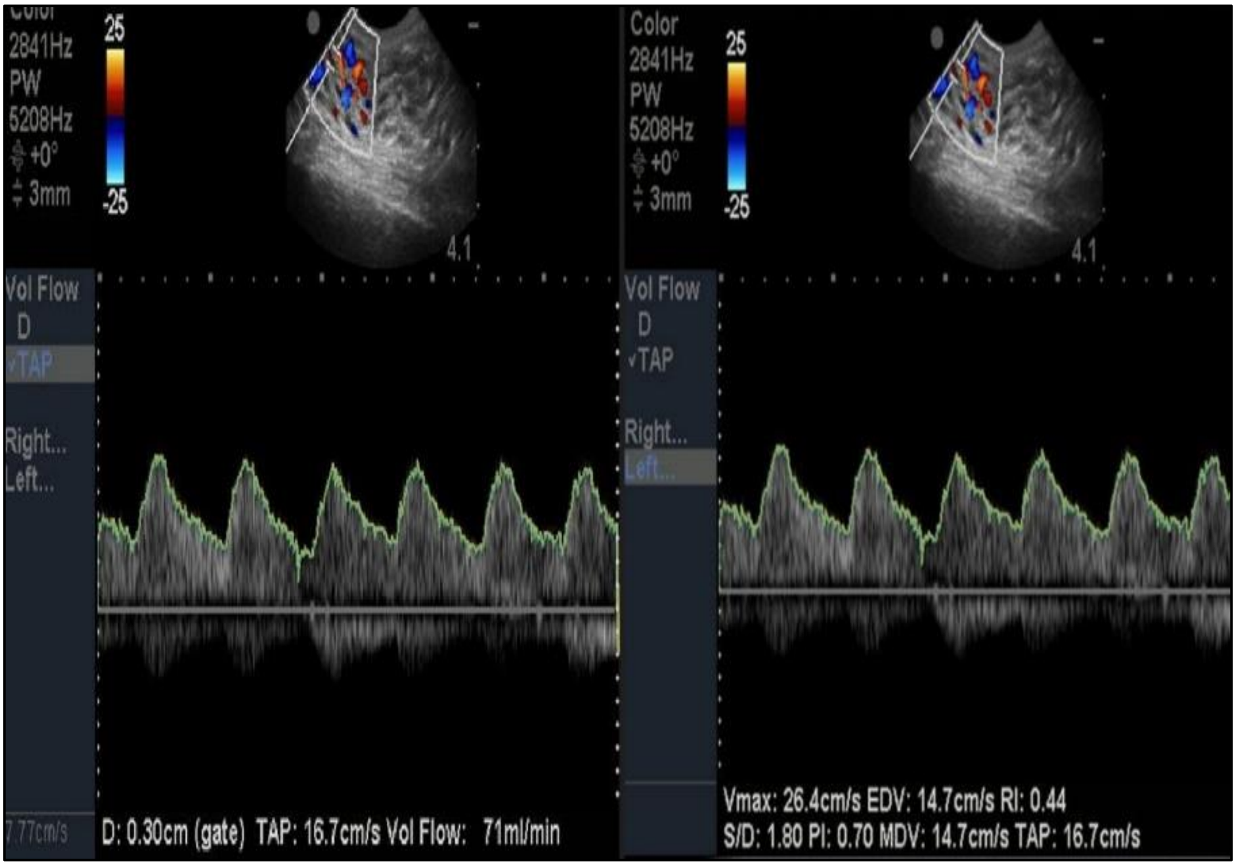

Figure 4. Color Doppler ultrasound images of supra-testicular artery in Akkaraman rams.

Table 10. Monthly PSV values measured by Doppler ultrasound in spermatic cord of control and PRP groups

Month	n	PSV ($cm \cdot s^{-1}$)				p-value
		Control		PRP		
		Mean±SE	Median	Mean±SE	Median	
July	16	9.60±0.70^{ae}	9.18	10.51±0.89^{aef}	10.30	0.503
August	15	10.52±0.43^{ac}	10.96	11.09±1.29^{acf}	10.15	0.497
September	14	14.34±0.78^{bdf}	14.37	15.00±1.10^{bcdg}	14.04	0.980
October	13	13.39±0.90^{bdf}	12.56	14.83±1.45^{abcdg}	13.24	0.695
November	12	16.09±1.56^{bf}	14.79	14.66±0.73^{cdg}	14.69	0.809
December	11	12.78±1.06^{bcd}	11.42	14.14±1.23^{cd}	15.32	0.579
January	10	13.56±0.52^{bdf}	13.20	12.14±0.99^{cg}	11.72	0.050
February	9	12.88±0.88^{adf}	13.96	15.04±1.32^{deg}	14.73	0.101
March	8	14.71±0.60^{ef}	14.75	14.17±1.69^{cdeg}	12.45	0.318
April	7	15.76±1.28^{af}	15.36	16.11±2.72^{fg}	13.89	0.841
Mean	115	13.21±0.35	13.20	13.66±0.42	13.21	0.702

-Differences between values carrying different lowercase letters (a-g) within the same column are statistically significant ($p<0.05$).

-Median values are presented for data that do not follow a normal distribution.

Table 11. Monthly EDV values measured by Doppler ultrasound in spermatic cord of control and PRP groups

Month	n	EDV ($cm \cdot s^{-1}$)				p-value
		Control		PRP		
		Mean±SE	Median	Mean±SE	Median	
July	16	6.94±0.66[ac]	6.24	7.01±0.60[a]	7.35	0.809
August	15	8.11±0.51[abc]	8.45	7.50±1.26[a]	6.61	0.080
September	14	10.36±0.73[bd]	9.93	10.44±0.91[bc]	9.55	0.742
October	13	8.69±1.09[abc]	9.18	9.64±0.65[abc]	9.37	0.449
November	12	9.19±0.91[abc]	9.00	10.20±0.76[abc]	10.70	0.468
December	11	8.66±0.73[abc]	8.63	10.11±0.91[c]	9.19	0.247
January	10	8.45±0.85[abc]	7.71	8.33±0.61[ab]	8.81	0.796
February	9	7.61±0.76[abc]	7.35	9.40±0.99[abc]	8.81	0.318
March	8	8.14±1.33[ab]	8.08	9.92±1.06[abc]	8.08	0.620
April	7	7.27±0.68[cd]	7.71	9.67±1.36[abc]	9.91	0.177
Mean	115	8.47±0.27	8.45	9.23±0.29	8.81	0.130

-Differences between values carrying different lowercase letters (a-d) within the same column are statistically significant ($p<0.05$).

-Median values are presented for data that do not follow a normal distribution.

Table 12. Monthly RI values measured by Doppler ultrasound in spermatic cord of control and PRP groups

Month	n	RI (PSV-EDV/PSV)				p-value
		Control		PRP		
		Mean±SE	Median	Mean±SE	Median	
July	16	0.78±0.21^{acd}	0.48	0.50±0.15	0.29	0.173
August	15	0.23±0.03^{be}	0.24	0.33±0.05	0.36	0.140
September	14	0.28±0.03^{bcf}	0.27	0.30±0.03	0.30	0.432
October	13	0.63±0.18^{cdf}	0.36	0.32±0.04	0.32	0.449
November	12	0.40±0.06^{bcdf}	0.42	0.29±0.06	0.29	0.173
December	11	0.46±0.16^{bcdf}	0.38	0.27±0.05	0.22	0.579
January	10	0.36±0.07^{bcdf}	0.35	0.30±0.04	0.30	0.730
February	9	0.61±0.18^{acdf}	0.40	0.35±0.07	0.31	0.097
March	8	0.46±0.11^{de}	0.41	0.29±0.04	0.27	0.209
April	7	0.54±0.03^{aef}	0.53	0.32±0.08	0.28	0.082
Mean	115	0.46±0.04	0.36	0.33±0.02*	0.30	**0.034**

-*Significant from control (p<0.05).

-Differences between values carrying different lowercase letters (a-f) within the same column are statistically significant (p<0.05).

-Median values are presented for data that do not follow a normal distribution.

Table 13. Monthly blood flow volume values measured by Doppler ultrasound in spermatic cord of control and PRP groups

Month	n	Blood flow volume (mL·min^{-1})				p-value
		Control		PRP		
		Mean±SE	Median	Mean±SE	Median	
July	16	27.80±2.94[a]	29.00	29.27±2.71[ad]	31.00	0.809
August	15	29.58±2.61[a]	33.00	33.20±5.26[ac]	30.00	0.456
September	14	38.92±3.34[b]	40.00	42.57±2.82[be]	42.50	0.595
October	13	33.00±2.29[ab]	34.00	43.17±3.61[abe*]	41.00	**0.023**
November	12	40.90±2.70[ab]	39.00	43.18±2.00[bce]	42.00	0.426
December	11	37.70±2.20[b]	38.50	45.40±4.20[bde]	43.50	0.353
January	10	35.89±2.64[ab]	35.00	35.67±1.66[abe]	35.00	0.931
February	9	39.43±3.36[ab]	40.00	44.43±2.95[de]	47.00	0.318
March	8	40.71±1.52[ab]	41.00	41.00±3.30[de]	40.00	0.710
April	7	39.60±3.06[ab]	36.00	44.83±6.07[de]	39.50	0.537
Mean	115	35.82±0.97	37.00	40.05±1.20	38.00	0.072

-*Significant from control ($p<0.05$).

-Differences between values carrying different lowercase letters (a-e) within the same column are statistically significant ($p<0.05$).

-Median values are presented for data that do not follow a normal distribution.

Discussion

A multitude of factors have been demonstrated to exert an influence on platelet count, including species, breed, age, sex, pregnancy status (Habibu et al., 2017), and nutritional status (Jin et al., 2019). Platelet counts exhibit variation according to animal species. For instance, equines have a platelet count of >100 million·mL^{-1}, while canines have a platelet count of >200 million·mL^{-1}. However, platelets in ruminants are even higher, with counts reaching >400 million·mL^{-1}. Furthermore, young animals exhibit higher platelet counts (Habibu et al., 2017& Cornell University College of Veterinary Medicine, 2024). In this study, blood samples taken monthly for 10 months were measured to determine the amount of platelets and growth factor levels in the PRP content to be administered to the animals. Despite fluctuations in platelet counts in whole blood across months, with the highest levels observed in July and August, the 10-month mean values remained within the standard range (422.89 million·mL^{-1}). A similar trend was observed in the platelet levels in PRP, which exhibited variation between months, peaking in July and August at 4.41 times the mean value of whole blood (1864.63 million·mL^{-1}). These observations align with the established literature, which reports that platelet levels in PRP are 3-5 times higher than those in whole blood (Sánchez-González et al., 2012& Karadağ Sarı, 2022). Furthermore, the elevated platelet levels observed in the animals in July and August can be attributed to the fact that these animals were of a young age, and platelet count is typically high in young individuals (Habibu et al., 2017& Cornell University College of Veterinary Medicine, 2024).

In this study, while significant differences in FGF1 values were observed between months in PRP samples, no such differences were detected in the levels of other growth factors (VEGF, IGF1, TGFβ and PDGF). The lowest FGF1 level was observed in July, when the animals were young, and the highest level was observed in March, when the animals were one-year-old. Although Fujii et al.,. (2019) reported a positive correlation between age and plasma FGF21 concentration, the reason for the changes in FGF1 levels between months in the present study is exactly unknown. In terms of average levels, it was observed that the PRP obtained in this study contained the highest level of IGF1, followed by PDGF, VEGF, TGFβ and FGF1, respectively. This suggests that following intratesticular injection of PRP, the IGF1 levels in the testis would be much higher in concentration compared to the other growth factors detected.

It has been reported that intratesticular PRP injections increase testosterone levels in the seminal plasma of rabbits (Abdulla et al., 2022), in oligoasthenoteratozoospermic men (Somova et al., 2021), and in rats with testicular damage induced using various models (Sekerci et al., 2017& Dehghani et al., 2019& Kutluhan et al., 2021). However, it is also suggested that there is no statistically significant change in testosterone levels in healthy rats (Dehghani et al., 2019) and diabetic rats (Rizal et al., 2020) receiving intratesticular PRP injection. Among the growth factors present in PRP, VEGF has been reported to enhance proliferation of Leydig cells in mice (Hwang et al., 2007), PDGFBB to affect the steroidogenesis mechanism of Leydig cells in adult rats in vitro (Risbridger, 1993), IGF1 to increase Leydig cells' response to LH in premature rats (Ohyama et al., 1995), and FGF to exhibit high affinity for Leydig cell membrane receptors in pigs (Sordoillet et al., 1992), thereby increasing testosterone secretion.

PRP has been known to contain high concentrations of various growth factors (Pavlovic et al., 2016). In this study, a significant increase in testosterone levels was observed within both the control and PRP groups in October compared to previous months, followed by a significant decrease in November, December, January, and February, and another significant increase in March and April. Testosterone production in rams exhibits a fluctuation pattern, increasing until the sexually active period after birth and then decreasing (Preston et al., 2012). These fluctuations in testosterone secretion are particularly evident between the ages of 2 and 14 months (Elmaz et al., 2007). Maksimovic et al.,. (2016) reported that testosterone levels in male lambs at 3 months of age (June) increased until 7 months of age (October), decreased in the following 4-5 months and increased again at 12-13 months of age (April). The monthly fluctuations in intra-group testosterone levels in the present study were consistent with previously documented findings (Elmaz et al., 2007& Maksimovic et al., 2016). The increase in intra-group testosterone concentrations observed in October could be explained by the fact that this month coincides with the peak of sexual activity during the breeding season or the attainment of puberty. The increases observed in March and April can be attributed to the animals reaching sexual maturity. With regard to the impact of PRP on plasma testosterone levels, the present study revealed a non-statistically significant numerical increase in the PRP groups in comparison to the control group in testosterone levels of all months except August, as well as in 10-month mean testosterone levels. Despite the observed numerical increases, PRP was found to have no significant effect on plasma testosterone levels. This result supports the findings of Dehghani et al., (2019) in healthy rats but does not align with those of Abdulla et al.,. (2022) in the seminal plasma of healthy rabbits.

The morphometric characteristics of the testes, which can be easily measured in young and adult animals, are among the most fundamental indicators used to evaluate ram fertility and select sire animals. In this study, it was determined that testicular length, thickness, width, volume, scrotal circumference, and scrotal thickness values increased in both the control and PRP groups in parallel with age. However, a decline was observed during the winter months, corresponding to December, January, and February, following October-November (the months coinciding with puberty), with increases resuming from March onwards. It has been reported that age and body weight in lambs are directly proportional to testicular dimensions and volume (Koyuncu et al., 2005). Furthermore, scrotal circumference measurements of 3-month-old male lambs in June gradually increase until they reached 7-8 months of age in October-November, decrease in December-February, increase again in March and continue in parallel afterwards (Maksimovic et al., 2016). Similarly, it has been suggested that scrotal circumference measurements in rams are at their lowest during the winter months (Kafi et al., 2004). The data obtained in this study regarding the intra-group morphometric values were consistent with the study of Maksimovic et al.,. (2016). Although testicular morphometric values are influenced by many factors, they mostly reach peak levels during the breeding season. This phenomenon may be attributed to the impact of daylight duration on neuro-hormonal mechanisms that regulate sexual functions (Chemineau et al., 2010). In support of this hypothesis, a decline in testosterone levels was observed during the winter months, concurrent with a decrease in testicular morphometric measurements. Conversely, no significant effect of PRP on testicular morphometric measurements was observed in this study. The precise mechanism underlying the significantly higher testicular length values in April and scrotal thickness values in December in the PRP group remains to be elucidated.

Testicular artery provides stable blood flow required for metabolic mechanisms and spermatozoon production (Bergh and Damber, 1993). Colour Doppler ultrasonography is a non-invasive tool necessary for analysing testicular hemodynamic (Samir et al., 2021). Recently, colour Doppler ultrasonography has been used to characterize testicular blood flow in farm animals such as rams (Hedia et al., 2019), goats (Ribeiro et al., 2020), and stallions (Pozor and McDonnell, 2004). Doppler measurement parameters of blood flow in the spermatic cord correlate with age, testosterone and oestrogen levels, testicular morphometric characteristics, semen quality, and fertility (Samir et al., 2021). The most important parameters related to blood flow are PSV, EDV, PI, RI and blood flow volume. Among these, PSV and EDV reflect arterial blood flow velocities. However, it has been reported that the measurement values of these two parameters are variable and inconsistent (Pozor and McDonnell, 2004). The RI reflects resistance to blood flow caused by the microvascular beds distal to the measurement region. The PI is designed to measure the pulsations in the waveform. RI and PI parameters are widely used to examine testicular blood flow perfusion (Biagiotti et al., 2002& Pozor, 2007& Zelli et al., 2013). In a study by Venianaki et al., (2023) on dogs, variations in Doppler measurement parameters were observed during pre-pubertal, pubertal, and post-pubertal periods, with reported differences in hemodynamic parameters of the testicular artery to meet the needs of the testicular parenchyma (for spermatogenesis) and epididymis (for spermatozoon maturation and storage) in parallel with age. Seasonal differences in testicular artery hemodynamic, particularly in RI and PI values, have been reported in Shiba goats, which do not show seasonal reproductive differences. This is attributed to adaptations to changes in ambient temperature and the regulation of arterial hemodynamic associated with thermoregulation (Samir et al., 2018). Hedia et al., (2019) observed that RI, PI, and flow volume were the most significant hemodynamic parameters in Doppler imaging and noted a significant relationship between high flow volume/low RI/low PI and high fertilization rates. These parameters reached their maximum values during the breeding season from October to March in rams. In this study, significant differences in Doppler measurement parameters were detected between months in both groups. Although these differences did not appear to be influenced by age, month, breeding season, or testosterone concentrations, flow volume and PSV values were found to be lowest in animals aged 3–4 months (July–August), with a significant increase in testicular blood flow starting from the 5th month (September).

This increase in testicular blood flow from the 5th month onward can be explained by the growing metabolic, oxidative, and hormonal demands of the testis as it enlarges, necessitating increased blood flow in the testicular artery.

PRP has been shown to significantly increase blood flow to deformed organs and tissues in many pathological conditions (Bir et al., 2009& Dhananjaya et al., 2018& Morad et al., 2021). PRP has also been reported to help alleviate dyspareunia symptoms in postmenopausal women by increasing urogenital blood flow and improving vaginal lubrication (Romashchenko et al., 2022). Significant increases in blood flow perfusion were observed in cats with cutaneous wounds up to the 25th day after PRP application, as assessed by Doppler ultrasound (Angelou et al., 2022). However, PRP was found to worsen renal blood flow in rats with renal ischemia-reperfusion (Martin-Sole et al., 2016) and had no effect on Doppler measurement parameters when injected into the corpus cavernosum of men with erectile dysfunction (Masterson et al., 2023). In this study, intratesticular PRP injection significantly increased blood flow volume in October. Testes require a high level of blood flow for active spermatogenesis and testosterone production (Damber et al., 1985). In the present study, in October, numerical but insignificant increment were detected in testosterone level in the PRP group compared to the control group. Moreover, statistically significant decreases in the 10-month average RI values were observed with PRP application. RI is an indirect measurement of resistance to arterial blood flow, used to evaluate vascular damage. RI is proportional to vascular resistance and compliance& when the vessel narrows and resistance to blood flow increases, RI also rises (Boote, 2003). Low RI values are positively correlated with blood flow, volume, and semen quality (Hedia et al., 2019).

Conclusion

The monthly intratesticular administration of PRP, which is highly rich in growth factors, to 2-month-old lambs over a period of 10 months did not result in a statistically significant increase in testicular morphometric measurements or testosterone levels. However, the 10-month mean RI value in the testicular artery was observed to decrease in response to PRP. This resulted in a modest increase in blood flow to the testis. Therefore, the administration of PRP to lambs at an early age may exert a slight beneficial influence on testicular development. However, further research is necessary to elucidate the effects of PRP on the microenvironment of testicular tissue in rams.

Conflict of interest: No conflict of interest declared.

For Studies with Ethics Committee Approval: The experimental phase of this study was carried out with the approval of Fırat University Animal Experiments Local Ethics Committee (Protocol No: 2020/05).

Obtaining Informed Consent: All participants provided written informed consent prior to data collection.

For Studies Conducted in More Than One Country: Ethical approval was obtained in all participating countries, and the research adhered to local regulations and the principles of the Declaration of Helsinki.

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Data Availability Statement: The data supporting the findings of this study are available from the corresponding author upon reasonable request after publication of the article. Corresponding author e-mail: aslihan.cakir@siirt.edu.tr

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