Testicular injection of Autologous Platelet Rich Plasma (PRP) to improve of semen parameters of goats

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Abstract

The present study has been designed to investigate wheather PRP effect on semen of goats. The study has been conducted on adult male goat at the, Al-Qadisiya Gouvernante Al Muhanawiah locality during the period extended from March 3 2022, to June 2 2022. Male goats were assigned to 5 equal groups and treated as follow: Control (C), T1: inject testes with PRP (0.25 ml), T2: injected with PRP (0.50 ml), T3: injected with PRP (0.75 ml), T5: just injected with needle . Male goats have been monitored throughout the experimental groups. At the end of each treated and control, male goat, the semen collection by the Elecrto ejacolater apparatus to evaluation of (volume, concentration, live abnormal and PH). The results of this study demonstrated there was a significant increase ($p<0.05$) in sperms concentration, sperms motility % and sperms live % after treated with PRP in T1, T2 and T3 groups as compared with T4 and control groups in 10th week, while there was a best result and a significant increase in sperms concentration in T2 group as compared with T1, T3, T4 and control group in 10th week. As well as the results showed a significant increase ($p<0.05$) in sperms concentration after treated with PRP in T1, T2 and T3 groups as compared with T4 and control groups in 12th week, while there was a best result and a significant increase in sperms concentration in T2 group as compared with T1, T3, T4 and control group in 12th week. Whereas the results showed a significant increase ($p<0.05$) in sperms concentration after treated with PRP in T1, T2 and T3 groups as compared with T4 and control groups in 14th week, while there was a best result and a significant increase in sperms concentration in T2 group as compared with T1, T3, T4 and control group in 14th week. And there was non-significant difference ($p > 0.05$) between Control and T4 groups. As for the time periods from the first week to the third week, they were without injections of anything, and there were no significant differences between the values for all groups. As for the 10th week, the 12th week, and the 14th week, there were significant differences between them in terms of values, especially in the T1, T2 and T3 groups, compared with the control group and the T4 group. Also demonstrated there was a significant decrease ($p<0.05$) in sperms abnormalities % after treated with PRP in T1, T2 and T3 groups as compared with T4 and control groups in 10th week, while there was a best result and a significant increase in sperms abnormalities % in T2 group as compared with T1, T3, T4 and control group in 10th week. As well as the results showed a significant decrease ($p<0.05$) in sperms abnormalities % after treated with PRP in T1, T2 and T3 groups as compared with T4 and control groups in 12th week. Whereas the results showed a significant decrease ($p<0.05$) in sperms abnormalities % after treated with PRP in T1, T2 and T3 groups as compared with T4 and control groups in 14th week. And there was non-significant difference ($p > 0.05$) between Control and T4 groups. As for the time periods from the first week to the third week, they were without injections of anything, and there were no significant differences between the values for all groups. As for the 10th week, the 12th week,

and the 14th week, there were significant differences between them in terms of values, especially in the T1, T2 and T3 groups, compared with the control group and the T4 group.

INTRODUCTION

Goat production plays an important role for the livelihoods of farmers in developing countries of subtropical regions. Goats contribute in economy, poverty alleviation and food security of rural households in terms of meat, milk, income, capital storage, savings, an insurance against emergencies and serving cultural purposes (Lebbie,2004).Goats disseminated all over the world due to their unique characteristics, such as their ability to withstand heat stress, endure prolonged water deprivation and adapt to adverse climatic and geophysical conditions where cattle and sheep can hardly survive (Abdel Aziz,2010). Reproduction in goats is commonly described as seasonal with differences in seasonality between breeds and locations, the onset and length of the breeding season in goats is dependent on a number of factors: latitude and climate, breed, physiological stage, presence of a male, breeding system but mainly photoperiod (Al-Atiyat,2014). Male fertility necessitates the generation of a sufficient number of adequately functional sperm that can pass through the female reproductive canal and penetrate the oocyte's zona pellucid, resulting in the formation of a viable embryo. Male infertility can be caused by a deficiency in any component of sperm function, including sperm concentration, motility, acrosome response, piercing the zona pellucid, and chromatin integrity (Al-Atiyat and Tabbaa,2009). Marini et al. (2016) evaluated the effect of PRP in vitro for bovine endometrial inflammation and obtained an important anti-inflammatory response in the evaluated cells, concluding that PRP should be considered a potential treatment for endometritis in vivo. Endometritis is characterized by an increased number of inflammatory cells associated with epithelial erosion and/or necrosis, and diffuse oedema of the endometrium. In most cases, the normal uterus is able to clear a bacterial infection efficiently. However, an uncontrolled infection (10%-20% of cows) may lead to chronic uterine inflammation. Jang et al. (2017) investigated PRP treatment for damaged endometrium, and the research group concluded that the intrauterine administration of autologous PRP stimulated and accelerated regeneration of the endometrium and decreased fibrosis in a murine model.

Materials and Methods

Semen evaluation

Macroscopic evaluation (volume, color, consistency, smell, impurities) of all ejaculates was performed directly after semen collection. The pH of each semen sample was measured with pH indicator-paper calibrated with whole numbers every 10 min for 1 h following collection. Concentration and sperm motion parameters were evaluated using computer assisted semen analysis on a plate warmed to 38 °C, negative phase contrast and × 10 objective at 3, 10, 15 and 30 min after collection. The amount of extender depended on the sperm concentration. For this purpose a commercial semen extender without animal proteins was used. Diluted semen samples were filled into Leja® counting chamber slides and directly a minimum of 2000 sperm cells were analysed.

Preparations of PRP

The most common way to prepare PRP involves centrifuging the blood sample. A vial of blood is placed in a centrifuge, where it is spun at intensely high speeds. The spinning causes the blood to separate into layers:

- Red blood cells, approximately 45% of blood, are forced to the bottom of the vial.

- White blood cells and platelets form a thin middle layer, called a buffy coat, which comprises less than 1% of the centrifuged blood.
- "Platelet-poor" plasma, or plasma with a low concentration of platelets, makes up the remaining top layer, about 55% of the centrifuged blood sample.

Once the centrifuge process is complete the doctor or medical technician will remove the vial from the centrifuge and prepare the PRP solution for injection. Centrifugation speed and time can vary.

Experimental design

Male gout were assigned to 2 equal groups (35 each) and treated as follow:

1. Control (C): without any things
2. T1: injected with PRP (0.25 ml).
3. T2: injected with PRP (0.50 ml).
4. T3: injected with PRP (0.75 ml).
5. T5: just injected without any things.

Male gout have been monitored throughout the experimental groups. At the end of each treated and control, male goat, the semen collection by the electronic enjuction apparatus to evaluation of (volume, concentration, live abnormal and PH).

Statistical Analysis:

Results were expressed as mean ± standard error of the mean (SEM). Comparisons were performed using one-way analysis of variance (ANOVA1). Differences were considered to be significant at the level of $P<0.05$. All statistical analysis were carried out using the (SPSS, Version,2010).

Results

Semen Parameters in Male Goat

Concentration of Sperms (million/ml).

Table, 1 demonstrated there was a significant increase ($p<0.05$) in sperms concentration after treated with PRP in T1, T2 and T3 groups (3.12 ± 0.02; 4.28 ± 0.09 and 3.63 ± 0.2) respectively, as compared with T4 and control groups (2.74 ± 0.02 and 2.88 ± 0.007) in 10th week, while there was a best result and a significant increase in sperms concentration in T2 group (4.28 ± 0.09) as compared with T1, T3, T4 and control group in 10th week. As well as the results showed a significant increase ($p<0.05$) in sperms concentration after treated with PRP in T1, T2 and T3 groups (3.13 ± 0.02; 3.93 ± 0.02 and 3.51 ± 0.02) respectively, as compared with T4 and control groups (3.51± 0.02 and 2.8 ± 0.01) in 12th week, while there was a best result and a significant increase in sperms concentration in T2 group (3.93 ± 0.02) as compared with T1, T3, T4 and control group in 12th week. Whereas the results showed a significant increase ($p<0.05$) in sperms concentration after treated with PRP in T1, T2 and T3 groups (3.02 ± 0.02; 3.68 ± 0.06 and 3.29 ± 0.02) respectively, as compared with T4 and control groups (2.61 ± 0.04 and 2.87 ± 0.006) in 14th week, while there was a best result and a significant increase in sperms concentration in T2 group (3.68 ± 0.06) as compared with T1, T3, T4 and control group in 14th week. And there was non-significant difference ($p > 0.05$) between Control and T4 groups. As for the time periods from the first week to the third week, they were without injections of anything, and there were no significant differences between the values for all groups. As for the 10th week, the 12th week, and the 14th week, there were significant differences between them in terms of values, especially in the T1, T2 and T3 groups, compared with the control group and the T4 group.

Table 1 The Effect of PRP on Sperm concentration 10*6 in Male Goat.

Groups	W1 W2 W3	W10	W12	W14
C	2.84 ± 0.01^{Aa}	2.88 ± 0.007^{Aa}	2.83 ± 0.01^{Aa}	2.87 ± 0.006^{Aa}
T1	2.73 ± 0.03^{Aa}	3.12 ± 0.02^{Bb}	3.13 ± 0.02^{Bb}	3.02 ± 0.02^{Ab}
T2	2.85 ± 0.07^{Aa}	4.28 ± 0.09^{Cb}	3.93 ± 0.02^{Cc}	3.68 ± 0.06^{Bd}
T3	2.76 ± 0.04^{Aa}	3.63 ± 0.2^{Db}	3.51 ± 0.02^{Db}	3.29 ± 0.02^{Cc}
T4	2.85 ± 0.05^{Aa}	2.74 ± 0.02^{Aab}	2.8 ± 0.01^{Aa}	2.61 ± 0.04^{Db}
LSD				0.171

C (Control): without any things, T1: injected with PRP (0.25 ml), T2: injected with PRP (0.50 ml), T3: injected with PRP (0.75 ml), T4: just injected without any things, W: Week

Percentage of Sperms Motility (%).

Table, 2 demonstrated there was a significant increase ($p<0.05$) in sperms motility % after treated with PRP in T1, T2 and T3 groups (78.25 ± 1.2; 91.75 ± 1.18 and 85.5 ± 0.28) respectively, as compared with T4 and control groups (69.5 ± 0.64 and 60.75 ± 2.17) in 10th week, while there was a best result and a significant increase in sperms motility % in T2 group (91.75 ± 1.18) as compared with T1, T3, T4 and control group in 10th week. As well as the results showed a significant increase ($p<0.05$) in sperms motility % after treated with PRP in T1, T2 and T3 groups (72.5 ± 0.95; 89.5 ± 0.28 and 76.75 ± 0.85) respectively, as compared with T4 and control groups (63.5 ± 0.64 and 61.75 ± 1.03) in 12th week, while there was a best result and a significant increase in sperms motility % in T2 group (89.5 ± 0.28) as compared with T1, T3, T4 and control group in 12th week. Whereas the results showed a significant increase ($p<0.05$) in sperms motility % after treated with PRP in T1, T2 and T3 groups (70.0 ± 0.7; 78.75 ± 0.7 and 76.0 ± 0.4) respectively, as compared with T4 and control groups (62.5 ± 0.64 and 58.25 ± 3.47) in 14th week, while there was a best result and a significant increase in sperms motility % in T2 group (78.75 ± 0.7) as compared with T1, T3, T4 and control group in 14th week. And there was non-significant difference ($p > 0.05$) between Control and T4 groups. As for the time periods from the first week to the third week, they were without injections of anything, and there were no significant differences between the values for all groups. As for the 10th week, the 12th week, and the 14th week, there were significant differences between them in terms of values, especially in the T1, T2 and T3 groups, compared with the control group and the T4 group.

Table 2 The Effect of PRP on Sperm motility % in Male Goat.

Groups	W1 W2 W3	W10	W12	W14
C	63 ± 0.8^{Aa}	60.75 ± 2.17^{Aab}	61.75 ± 1.03^{Aa}	58.25 ± 3.47^{Ab}
T1	65.83 ± 0.73^{Aa}	78.25 ± 1.2^{Bb}	72.5 ± 0.95^{Bc}	70 ± 0.7^{Bc}
T2	67.83 ± 0.73^{Ba}	91.75 ± 1.18^{Cb}	89.5 ± 0.28^{Cb}	78.75 ± 0.7^{Cc}
T3	67.08 ± 0.62^{Ba}	85.5 ± 0.28^{Db}	76.75 ± 0.85^{Dc}	76 ± 0.4^{Cc}
T4	68.16 ± 0.64^{Ba}	69.5 ± 0.64^{Ea}	63.5 ± 0.64^{Ab}	62.5 ± 0.64^{Db}
LSD		3.366		

Percentage of sperms abnormalities (%)

Table, 3 demonstrated there was a significant decrease ($p<0.05$) in sperms abnormalities % after treated with PRP in T1, T2 and T3 groups (7.72 ± 0.10; 6.72 ± 0.16 and 7.77 ± 0.16) respectively, as compared with T4 and control groups (8.62 ± 0.08 and 8.32 ± 0.12) in 10th week, while there was a best result and a significant increase in sperms abnormalities % in T2 group (6.72 ± 0.16) as compared with T1, T3, T4 and control group in 10th week. As well as the results showed a significant decrease ($p<0.05$) in sperms abnormalities % after treated with PRP in T1, T2 and T3 groups (6.95 ± 0.10; 7.12 ± 0.14 and 7.80 ± 0.14) respectively, as compared with T4 and control groups (8.47± 0.18 and 8.45 ± 0.18) in 12th week. Whereas the results showed a significant decrease ($p<0.05$) in sperms abnormalities % after treated with PRP in T1, T2 and T3 groups (7.87 ± 0.14; 7.97 ± 0.08 and 8.22 ± 0.02) respectively, as compared with T4 and control groups (8.35 ± 0.13 and 8.70 ± 0.04) in 14th week. And there was non-significant difference ($p > 0.05$) between Control and T4 groups. As for the time periods from the first week to the third week, they were without injections of anything, and there were no significant differences between the values for all groups. As for the 10th week, the 12th week, and the 14th week, there were significant differences between them in terms of values, especially in the T1, T2 and T3 groups, compared with the control group and the T4 group.

Table 3 The Effect of PRP on Sperm abnormality % in Male Goat.

Groups	W1 W2 W3	W10	W12	W14
C	8.4 ± 0.06 ^{Aab}	8.32 ± 0.12^{Aa}	8.45 ± 0.06^{Aab}	8.7 ± 0.04^{Ab}
T1	8.27 ± 0.03^{Aa}	7.72 ± 0.10^{Bb}	6.95 ± 0.10^{Bc}	7.87 ± 0.14^{Bb}
T2	8.12 ± 0.06^{Aa}	6.72 ±0.16^{Cb}	7.12 ± 0.14^{Bc}	7.97 ± 0.08^{Ba}
T3	8.11 ± 0.13^{Aab}	7.77 ± 0.16^{Ba}	7.8 ± 0.14^{Cab}	8.22 ± 0.02^{Cb}
T4	8.22 ± 0.11^{Aa}	8.62 ± 0.08^{Ab}	8.47 ± 0.18^{Aab}	8.35 ± 0.13A^{Cab}
LSD	0.355			

Percentage of Live Sperms (%)

Table, 4 demonstrated there was a significant increase ($p<0.05$) in sperms live % after treated with PRP in T1, T2 and T3 groups (70.66 ± 0.36; 89.42 ± 0.14 and 82.53 ± 0.44) respectively, as compared with T4 and control groups (67.74 ± 0.58 and 69.35 ± 1.44) in 10th week, while there was a best result and a significant increase in sperms live % in T2 group (89.42 ± 0.14) as compared with T1, T3, T4 and control group in 10th week.

As well as the results showed a significant increase ($p<0.05$) in sperms live % after treated with PRP in T1, T2 and T3 groups (71.66 ± 0.59; 80.82 ± 1.09 and 76.42 ± 0.43) respectively, as compared with T4 and control groups (66.11 ± 0.46 and 68.82 ± 0.55) respectively, in 12th week, while there was a best result and a significant increase in sperms live % in T2 group (80.82 ± 1.09) as compared with T1, T3, T4 and control group in 12th week. Whereas the results showed a significant increase ($p<0.05$) in sperms live % after treated with PRP in T1, T2 and T3 groups (68.75 ± 0.4; 74.79 ± 0.89 and 71.40 ± 0.4) respectively, as compared with T4 and control groups (66.16 ± 0.36 and 67.17 ± 0.46) respectively, in 14th week, while there was a best result and a significant increase in sperms live % in T2 group (74.79 ± 0.89) as compared with T1, T3, T4 and control group in 14th week. And there was non-significant difference ($p > 0.05$) between Control and T4 groups. As for the time periods from the first week to the third week, they were without

injections of anything, and there were no significant differences between the values for all groups. As for the 10th week, the 12th week, and the 14th week, there were significant differences between them in terms of values, especially in the T1, T2 and T3 groups, compared with the control group and the T4 group.

Table 4 The Effect of PRP on Sperm live % in Male Goat.

Groups	W1	W2	W3	W10	W12	W14
C	65.63 ± 0.16^{Aa}			69.35 ± 1.44^{Ab}	68.82 ± 0.55^{Bbc}	67.17 ± 0.46^{ADc}
T1	65.87 ± 0.22^{Aa}			70.66 ± 0.36^{Ab}	71.66 ± 0.59^{Bb}	68.75 ± 0.4^{Ac}
T2	66.39 ± 0.41^{Aa}			89.42 ± 0.14^{Bb}	80.82 ± 1.09^{Cc}	74.79 ± 0.89^{Bd}
T3	65.98 ± 0.2^{Aa}			82.53 ± 0.44^{Cb}	76.42 ± 0.43^{Dc}	71.4 ± 0.4^{Cd}
T4	66.05 ± 0.45^{Aa}			67.74 ± 0.58^{Db}	66.11 ± 0.46^{Eab}	66.16 ± 0.36^{Dab}
LSD				1.674		

Discussion

The effects of testicular platelet-rich plasma (PRP) injection on the hormone and sperm parameters of goats were investigated. This study has four experimental groups (T1 through T4) and one control group. Each group was studied for fourteen weeks. The control groups did not get injections. In T1, goats received PRP (0.25 ml) injections. PRP was injected into the T2 (0.50 ml). 0.75 ml of PRP was injected into the T3. Simultaneously, the T4 was injected without any additional substances. The major objective of this study is to determine how intra-testicular PRP injection affects sperm quality. Over 14 weeks, the concentration of spermatozoa in the PRP group increased by a statistically significant amount. In addition, a considerable rise in overall motility, progressive motility, liveliness, hyperactivity, and normal morphology in the eighth and tenth weeks demonstrated the efficacy of PRP therapy. These results supported Sfakianoudis et al. (2019), which demonstrated that PRP growth factors could enhance parenchymal perfusion and stimulate testicular cells to produce more and better spermatozoa. However, PRP injections increase testicular function in tested animals. However, PRP therapy can increase their viability and motility (Valeriya et al., 2014).

The study demonstrated a significant increase ($p<0.05$) in sperm concentration after being treated with PRP in T1, T2 and T3 groups. There was also the best result and an increase in sperm concentration in the T2 group compared to the control group. This study demonstrated a significant increase ($p<0.05$) in sperm motility% after being treated with PRP in T1, T2 and T3 groups. There was the best result and significant increase in the motility of the T2 group (91.75 ± 1.18) compared with other groups (T4 and control) in the 10th week. The results demonstrated there was a significant decrease ($p<0.05$) in sperm abnormalities% after being treated with PRP in T1, T2 and T3 groups (7.72 ± 0.10) as compared with T4 and control groups (8.62 ± 0.16) in 10th week.

Previous research (Abdulla, Rebai, et al., 2022) determined that evaluating the effect of a compound on testicular function in bucks should be postponed until after conception, as spermatogenesis continues for another 48–52 days before the production of mature spermatozoa and the epididymis requires an additional 10–14 days. During male adolescence, testosterone plays a vital role in initiating and maintaining sperm production and differentiation of spermatids (El-Sherbiny et al., 2022). At four time periods, the effects of intratesticular PRP injection on

testosterone, Vascular endothelial growth factor (VEGF) levels in the seminal plasma were evaluated in male gout (Dehghani et al., 2019). Since a previous study has demonstrated that PRP injection stimulates Leydig cells to increase testosterone synthesis, it is not surprising that testosterone levels were elevated after a few weeks (Rizal et al., 2020). VEGF promotes meiosis and cell proliferation during spermatogenesis by promoting the formation of new blood vessels, which in turn facilitates the transport of nutrients to the testicular parenchyma and the subsequent maturation of spermatozoa (Abdulla, Rebai, et al., 2022). Consistent with previous findings that VEGF of PRP promotes germ cell proliferation, lifecycle maintenance, and apoptosis suppression, our investigation demonstrated that PRP intra-testicular injection increased the VEGF concentration in seminal plasma (Abdulla, Rebai, et al., 2022; Bigliardi et al., 2018; Dehghani et al., 2019). In addition, the results of this study support the study of Sekerci et al. (2017), who suggested that intra-testicular injection of PRP may increase the level of Insulin-like growth factor 1 (IGF-1) in seminal plasma at four weeks, which correlates with improved sperm parameters. This is likely due to the biological role of IGF-1 in reducing the apoptosis of germ cells and increasing density, as information by Bigliardi et al. (2018), Ozcan et al. (2020) indicated that testis development is dependent on the activation of both insulin and IGF-1. Studies (El-Sherbiny et al., 2022; Hermilasari et al., 2020; Sfakianoudis et al., 2019) revealed that PRP injection could lower IGF-1 synthesis in the testes by decreasing levels of growth hormone-releasing hormone in the hypothalamus, which would subsequently influence growth hormone production in the anterior pituitary.

In this study, the tested goats benefit from the preventive benefits of PRP because it increases antioxidant defence, decreases neutrophil infiltration, and decreases oxidative stress. According to Somova et al. (2021), Platelet-rich plasma (PRP) injected directly into the testes between weeks 6 and 10 significantly improved spermatogenesis and, consequently, the quality of the spermatozoa generated compared to the control group. In addition, the findings of Ozcan et al. (2020) indicated that the evaluation of PRP's effect on testosterone, VEGF, and IGF1 in seminal plasma and sperm parameters revealed, among other things, that PRP possessed a variety of characteristics that may have beneficial effects on spermatozoa regeneration in healthy bucks. Due to limitations, we could only conduct experiments with a small sample size; therefore, further funds are required to collect more data. (Abdulla et al., 2022). Spermatogenesis is the process by which spermatogenic and Sertoli cells create spermatozoa in the epithelium of the seminiferous tubules. Without Sertoli cells, food and growth nutrients cannot reach developing spermatogenic cells. Reorganisation of the spermatogenic layer and reproduction of spermatozoa in the testicular lumen are histological indicators of testicular damage repair following injection of PRP (Hermilasari et al., 2020). Rizal et al. (2020) demonstrated that treatment of PRP significantly increases the number of spermatogenic cells (spermatogonia, spermatocyte, spermatid) and the thickness of seminiferous tubules. Since glucose and gonadotropin levels were believed to have had a role, we hypothesised that this mechanism was at work. It is feasible but unlikely that PRP increases testosterone levels in tested animals. In a diabetic state, a lack of insulin stimulation on Leydig cells reduces the expression of the enzymes involved in steroidogenesis, inhibiting steroidogenesis (Somova et al., 2021). Since PRP contains many growth factors that stimulate angiogenesis, cell proliferation, and differentiation, reduce pro-apoptotic Bcl-2

gene expression, and inhibit apoptosis, it is currently widely used for tissue regeneration.

Following Sekerci et al. (2017), the growth factors in PRP help animals caused by streptozocin, resulting in improved cell regeneration and testicular damage recovery. PRP inhibits caspase-3 expression by activating Phosphoinositide 3-kinases (PI3K) to prevent excessive ROS production, which has a restorative effect on NFkB activation. PRP contributes to the restoration of testicular function in tested animals. We could only conduct a small-scale experiment in this study due to limited resources; future studies will benefit from a bigger sample size. Since all animals were housed in the same habitat and fed the same meal, seasonal variations in fresh semen sperm parameters cannot be linked to dietary variances. Nonetheless, sperm pathology increased somewhat during the dry season, which may have been due to the greater air temperatures that occurred during that time. Similarly, Widiyono et al. (2017) discovered comparable outcomes in the sperm pathology of goat sperm. It is commonly acknowledged that exposure to high temperatures promotes alterations in the seminiferous epithelium, which have detrimental effects on sperm quality. Experimentally induced increases in testicular temperature greatly exacerbate sperm pathology, and spermatogenesis can be completely halted by an increase in the normal testis' temperature, as observed in cryptorchid animals, or experimentally induced heat-stressed testes (Dehghani et al., 2019). A temperature increase of 1 or 2 degrees Celsius for eight hours can drastically disrupt the process of spermatogenesis. However, the extent of the harm depends on how long the testes were exposed to heat (Dehghani et al., 2019). According to El-Sherbiny et al. (2022), cooling sperm samples to 4°C significantly decreased the number of motile cells and the vitality of the samples during the cooling duration in both seasons. Sperm can be killed by heat shock during cooling. However, this danger can be avoided by cooling diluted sperm at a pace of 0.05oC/min to 4°C and adding phospholipid-containing extenders (Valeriya et al., 2014).

Further, El-Sherbiny et al. (2022) hypothesise that differences in cooling duration between the dry and rainy seasons are related to biochemical alterations detected in seminal plasma at the two times, as there were no significant differences in sperm motility and vigour of semen collected prior to cooling. We discovered that dry-season seminal plasma included much lower concentrations of phosphate, magnesium, citric acid, fructose, and total proteins than wet-season plasma. Valeriya et al. (2014) observed seasonal changes in the biochemical components of goat sperm. It is common knowledge that minerals are required for electrolytic equilibrium and the preservation of goat sperm. These factors govern the enzymes, membrane proteins, second messengers, receptors, and energy metabolism of reproductive systems. Because phosphatidylcholines (lecithins), lipoprotein yolk, and milk casein are all advantageous to spermatozoa during heat stress, they are typically found in sperm extenders (Sills, 2021). Sperm cells can be destroyed if goat sperm is diluted with egg yolk-containing extenders. Comparing the motility rate after two hours of chilling in the dry and rainy seasons reveals this. Increased phospholipase A2 activity in seminal plasma is thought to be responsible for a decline in sperm motility and vigour that coincides with the dry season and results in a significant increase in motility rate. Even when the temperature decreases or freezes, the phospholipases in seminal plasma have a negative effect. Tiras (2020) discovered that the quantity of viable sperm reduced linearly with the storage period. As previously determined, the presence of phospholipase in seminal goat plasma does not promote its preservation when chilled or frozen. The

modified Johnsen scoring system is reliable for evaluating spermatogenesis. These findings imply that intra-testicular PRP treatment enhances spermatogenesis. In the past, it was discovered that growth factors enhance spermatogenesis and suppress apoptosis (Sekerci et al., 2017).

Kutluhan et al. (2021) believe that because PRP is rich in growth factors, it may positively influence spermatogenesis. Platelet-rich plasma (PRP) enhances spermatogonia, primary spermatocyte counts, and sperm motility, according to research. Growth factors regulate germ cell proliferation and differentiation. Incorporating growth factors into the PRP decreases the time necessary for the various phases of meiotic division. The development of spermatozoa is enhanced under specific conditions. PRP did not affect the amount of interstitial tissue, the length of germinal epithelium, or the number of Leydig and Sertoli cells (Kutluhan et al., 2021). Our findings imply that intra-testicular PRP injection during detorsion may have favourable effects on Leydig cell dysfunction, as LH levels were lower and total testosterone levels were higher in the PRP group. According to previous studies, PRP's favourable effect on male fertility may fade over time (Dehghani et al., 2019). The positive benefits of PRP on spermatogenesis may require maintenance treatment with repeated dosing. However, the ideal dose and dosing interval for PRP application has yet to be found. Despite the beneficial effects of PRP on spermatogenesis and hormone production in the model, additional experimental and clinical research is necessary to advance our understanding of this subject.

Nonetheless, this analysis contains a few shortcomings. The histological research did not let us evaluate particular testicular cells, such as the Leydig cell; nevertheless, the hormonal data provided some insight into the Leydig cell's function (Dehghani et al., 2019). Moreover, we could not compare the groups regarding apoptosis, sperm morphology, fertility, or DNA damage status of spermatogenic cells. This is the fundamental limitation of our study. Using a single model, we learned more about the influence of RPR on reproductive hormone status. Fourth, we utilised the literature-recommended PRP dose, and it remains uncertain what the appropriate PRP dose and interval should be (Dehghani et al., 2019; Kutluhan et al., 2021; Nazari et al., 2022; Rizal et al., 2020; Sfakianoudis et al., 2019; Tiras, 2020; Widiyono et al., 2017). In addition, autologous PRP was not employed since the small blood volume of the tested animals made its application difficult. Although PRP is normally considered an autologous agent, we prepared PRP from the donor group in this study. Consequently, homologous intra-testicular PRP may provoke an immune response; however, a recent study by Somova et al. (2021) indicated that the benefits of homologous PRP outweighed the immunological effects. The oxidative stress levels of patients could not be measured. The average sperm motility of Kacang goats was between 60.0% and 83.3% after the feeding session. Although sperm motility was comparable during the times of feed restriction and re-alimentation, neither the feeding duration nor the feed therapy had a statistically significant effect on sperm motility. These findings confirm earlier studies on pigs, which revealed that food quantity had no discernible effect on sperm motility levels. In addition, the sperm motility levels of pigs fed ad libitum for weeks and then limited for weeks did not differ. Although Ozcan et al. (2020) discovered that the biochemical composition of sperm is associated with sperm motility and vitality, likely, the restricted feeding-refeeding in this study did not significantly modify these parameters. Sperm includes a high quantity of fructose, which provides spermatozoa with energy. A decrease in energy or feed intake decreases the fructose content of ejaculation

and the motility of sperm, according to research on the diets of rams. As demonstrated in our previous study with Kacang goats, blood chemistry parameter values were maintained within the physiological reference value range for these goats even after four weeks of feeding at 50% of full nutrition (Widiyono et al., 2017). Consequently, it is essential to study how to feed restriction and subsequent eight-week refeeding change the biochemical composition of goat sperm in Kacang breeds (Widiyono et al., 2017). Pathomorphological and morphometric investigations demonstrated that the PRP treatment restored the microstructure of the testicles of the experimental animals. Sperm production restarted, and the quantity and function of Sertoli cells recovered to normal levels (Sills, 2021; Tohidnezhad et al., 2011). The seminiferous tubule duct width had expanded to maintain testis levels, and multiple ducts held numerous newly generated sperm. This research indicates that PRP can restore normal gonad morphology and function after experimental toxic injury. This result is certainly due to the functioning of growth factors released from platelet granules after PRP injection. Among these growth factors are epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), and fibroblast growth factor (FGF), among others (Abdulla et al., 2022; El-Sherbiny et al., 2022; Kutluhan et al., 2021; Sfakianoudis et al., 2019). This outcome is attributable to the following characteristics of these physiologically active substances: stimulation of cell growth and differentiation, augmentation of angiogenesis and collagen synthesis, antiapoptotic effect, acceleration of cell migration, and others. Hypogonadism and diminished fertility are side effects of cancer chemotherapy; hence, patients undergoing this treatment are frequently advised to harvest samples of their seminiferous epithelium for cryopreservation and future treatment from a sperm bank (Hermilasari et al., 2020; Nazari et al., 2022; Ozcan et al., 2020; Salama et al., 2022; Tiras, 2020). PRP can help these individuals avoid this taxing and costly procedure. Therefore, it is reasonable to assume that platelet-rich plasma therapy effectively mends gonads damaged by toxicity.

Platelet-rich plasma (PRP) is an endogenous, autologous agent containing abundant growth factors and cytokines. Specifically, it has been utilised in the field of regenerative medicine. Both pro- and anti-inflammatory mediator levels have been shown to vary (Sills, 2021). PRP has demonstrated efficacy in treating various urological disorders, including erectile dysfunction, disease, and urethral stricture, because of its anti-inflammatory and regenerative qualities. Somova et al. (2021) conducted the first investigation into the effect of PRP on damage and hormone production in the animal. This experimental study proved the therapeutic effects of PRP on ionising radiation-induced oxidative stress, testicular histopathology, and hormone production. In tested animals with testicular atrophy produced by busulfan, intra-testicular PRP injection favoured total testosterone levels (Nazari et al., 2022).

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