Testicular Injection of Autologous Platelet-Rich Plasma (PRP) to Enhance the Sperm Parameters in Rabbit

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ABSTRACT

This study aims to determine the perfect time for semen collection after intra–testicular platelet-rich plasma to get good and improved semen. Twenty male New Zealand rabbits were used, about 10 to 12 months aged, which randomly divided into two equal groups; the control group and the PRP group which was enriched with 200 µl (4000 × 109 platelets/1 µl) single-dose by intra-testicular injection. The Laboratory analysis of all animals continued at four-time points (4thwk, 6thwk, 8thwk and 10thwk) by measuring the concentration of the Testosterone, Vascular Endothelial Growth Factor (VEGF) and Insulin-like Growth Factor-1 (IGF-1) in the seminal plasma by ELISA technique. Furthermore, the semen analyses which applied by the Computer Assisted Sperm Analysis (CASA) device. The findings of testosterone, VEGF levels and IGF-1 levels were significantly ($p<0.01$ & $p<0.05$) higher in the 4th wk and 6th wk in the PRP group vs. control group. Moreover, the PRP treatment significantly ($p<0.01$) increased the sperm parameters like concentration, total motility, progressive motility, hyperactivity, vitality and normal sperm morphology, especially after 8 to 10 weeks. The our research showed as the PRP intra-testicular injection can improve the testes' function after 8-10 weeks. This might lead to a novel possible treatment for oligoasthenoteratozoospermia.

Keywords: Platelet-rich plasma (PRP), Rabbit, Sperm Parameters, Testis Function

Introduction

Sperm production is dependent on adequate testicular activity, which influenced by hormones, growth factors and genetics. The process of spermatogenesis begins during puberty and continues throughout an adult male's life, resulting in continued sperm development and future fertility [1].

Platelet-Rich Plasma (PRP) is a type of autologous plasma with a higher platelet count than normal [2]. The PRP induces the release of several growth factors, such as vascular endothelial growth factor (VEGF), Insulin-like Growth Factor-1 (IGF-1), Fibroblast Growth Factor (FGF) and Epidermal Growth Factor (EGF). These factors stimulate tissue

regeneration by releasing biological micro molecules at the injection site [3, 4]

The use of the PRP in reproduction seems to be a reliable treatment choice with a scope of the possible positive effects of new therapies developing in the medical sector [5, 6]. At the reproductive level, many studies showed that intra-testicular PRP injection is promising in the treatment of unexplained infertility in humans [7].

The PRP enhances the testicular function by reducing the production of the Malondialdehyde and Interleukin-6 levels in rats. In addition, PRP treatment of rats poisoned by busulfan leads to enhancing both functional and structural disorders of the testes [8]. Yet, the application of the PRP was a success in the restoration of the male mice gonad in toxic conditions [9].

There are several explanations for the positive effect of PRP on the stimulation of sperm production; maybe it improves Leydig cell proliferation and testicular hormone production [10, 11]. Also, the PRP can activate the various forms of growth factors for instance VEGF and IGF-1 that lead to stimulating the multiple biologically active proteins that promoted proliferation, growth, differentiation of cells, and angiogenesis [12].

The goal of this study is to clarify the effect of intra-testicular injection of the PRP on sperm parameters such as concentration, motility, progressive sperm motility, sperm hyperactivity, vitality and morphology at four-time points ($4th^{wk}$, $6th^{wk}$, $8th^{wk}$ and $10th^{wk}$). In addition, we sought to confirm those effects by estimating the concentration of testosterone, VEGF and IGF-1 in seminal plasma. So that, it helps reveal the best time of semen collection to gate improved semen after injecting platelet-rich plasma into the testicle.

Materials and Methods

Animals and Study Design

In these experiments, we dealt with all animals under certain rules and regulations (whether feeding, watering, or injecting the PRP, this study done according to the Laboratory Animal Guide [13]. The Animal Care Unite of a college of Veterinary Medicine, University of Al-Qadisiyah, had ratified the experiments.

The study duration is from September 2020 to March 2021. We selected twenty healthy New-Zealand rabbit males aged 10-12 months and weighing 1.80-2.60 kg. All animals were confirmed to be free of any visible abnormalities of the reproductive organs; all bucks were kept under standard environmental conditions, fed with nourishing fodder, hay and root crops. During adaptation period of two weeks, the rabbits taught to service an artificial vaginal delivery system. These animals were separated into two equal groups at random; the control group (n=10) which was kept without any injection, and the PRP group (n=10) which was treated with autologous PRP.

PRP preparation

From each animal of the PRP group, a total of 10 mL of whole blood was collected, Then, a (3.2%) sodium citrate by 9/1 ratio was added, this mixture was added to the PRP gel tube with the Activator. (Biozek Medical®, Laan van de Ram, Bulgaria), and centrifugation as per the manufacturer's recommendations. The PRP was collected (2ml) drawn into the Eppendorf tube and kept at -80°C for subsequent use. Each PRP sample was divided into two tubes, the first tube was used to determine the platelet count by the auto-hematology analyzer (GKTV, Shenzhen Genius Electronics, China), as a final measure, the average value of three measurements was recorded. The second tube of PRP was used for intra-testicular injection as described [10]. The concentration of the PRP in this study was (4000×10^9 platelets/1 microliter) 200-µl single-dose injection into each testis parenchyma of PRP group animals, by inserting a sterile needle gauge 21 along the longitudinal axis and pushing the fluid with the withdrawal process [8].

Semen collection

The semen samples were collected by disposable artificial vagina device pre-filled with warm water (40°C), then transferred immediately to the research lab, and maintained at (38ºC). The sperm concentration (10^6/ml semen) was calculated by direct cell

count using the Hemocytometer (MARIENFELD®, Neubauer-Improved Platelet Counting Chamber, USA), by light microscope 400x according to the company guide. The semen samples collected at four-time points (4thwk, 6thwk, 8thwk and 10thwk), to apply the lab experiments.

ELISA technique

Applied the Enzyme-Linked Immunosorbent Assay (ELISA) to analyze the testosterone, VEGF and IGF-1 levels using (Elisys Uno®, Fully Automated ELISA Analyzer, HUMAN Co, Germany), with rabbit ELISA kits (Testosterone, VEGF and IGF-1, My BioSource Co., Alaska, USA), by double centrifugation (1,800 rpm/10 minutes) to remove sperm cells as per manufacturers recommendations.

CASA analysis

Applied the Computer Assisted Sperm Analysis (CASA) device to semen analysis at same those four points by (CEROS II®, Animal Semen Analysis, Zeiss, IMV-Technologies Co. France), with sperm chamber (Leja products BV ® 4 Chamber Slides depth 20 µm, IMV-Technologies Co. France), to determine the percent of total motility, progressive motility (VSL ≥ 25 µm/s + VSL < 25 µm/s), sperm hyperactivity (VCL >35 µm/s+ ALH >2.5 µm/s +STR >85), and vitality (live sperm) [14, 15]. Furthermore, the spermatozoa morphology index assessed by calculating the percent of the normal index of the head, neck and tail using the staining kit (SoermFunc®, Diff-Quik, China) by the manufacturer's recommendations.

Vocabulary: VSL (Straight-line velocity) = STR x VAP ,STR=Straightness of the path velocity (VSL/VAP), VAP; Average path velocity, VCL; Curvilinear velocity, ALH; Amplitude of Lateral Head displacement, VAP; Average path velocity

Statistical Analysis

The results (mean + standard error of the mean) of each parameter, the variance evaluated by repeated measures ANOVA (the time points were taken as factors). The statistical significance was determined as follows: $p<0.05$ and $p<0.01$. The SPSS/PC program was used to examine all of the variables by IBM® SPSS® statistical software 27.0, 2020; for Windows (SPSS Inc., Chicago, IL, USA) [16].

Results

ELISA results

The testosterone concentration (ng/ml) in seminal plasma of PRP group animals was significant ($p<0.01$) higher in the 4th wk (8.53± 03) and 6th wk (8.39±0.27) in comparison with a control group (6.22±0.33, 6.23 ±0.51) respectively, then it's back to normal concentration in 8th and 10th weeks. The VEGF level (ng/ml) was highly significant ($p<0.01$) in the PRP group in the 4th wk and 6th wk (17.59 ± 0.64, 19.48 ± 0.78) respectively, in comparison with the control group (10.94±0.57, 10.87±0.45) respectively. The IGF-1 level (ng/ml) was significant ($p<0.05$) in the PRP group at the 4th wk (15.29±0.49) in comparison with the control group (12.55±0.59). (Table 1)

Table 1. The ELISA results of Testosterone (TH), VEGF and IGF-1 (ng/ml) in seminal plasma of control and PRP groups at the four-time points of male rabbit's bucks (Mean + SEM).

Factors	4thwk		6thwk		8thwk		10thwk		P-value
	Control	PRP	Control	PRP	Control	PRP	Control	PRP	
TH	6.22±0.33	8.53±03	6.23±0.51	8.39±0.27	6.04±0.4	6.74±0.25	6.003±0.36	5.81±0.33	0.000**
VEGF	10.94±0.58	17.59±0.64	10.87±0.45	19.48±0.78	11.38±0.63	13.29±0.4	11.19±0.58	12.16±0.68	0.000**
IGF-1	12.55±0.59	15.29±0.49	12.18±0.38	13.52±1.09	12.76±0.81	12.64±0.45	12.47±0.7	13.49±0.85	0.816*

PRP; Platelet-rich plasma, SEM; Standard error of the mean, TH; Testosterone hormone, VEGF; Vascular Endothelial Growth Factor, IGF-1; Insulin-like Growth Factor-1. ** In the same rows indicate significant statistical differences (P <0.01) between the control group vs. PRP group at the different time points. * Indicate significant statistical differences (P <0.05) between the control group vs. PRP group at the different time points.

CASA Results

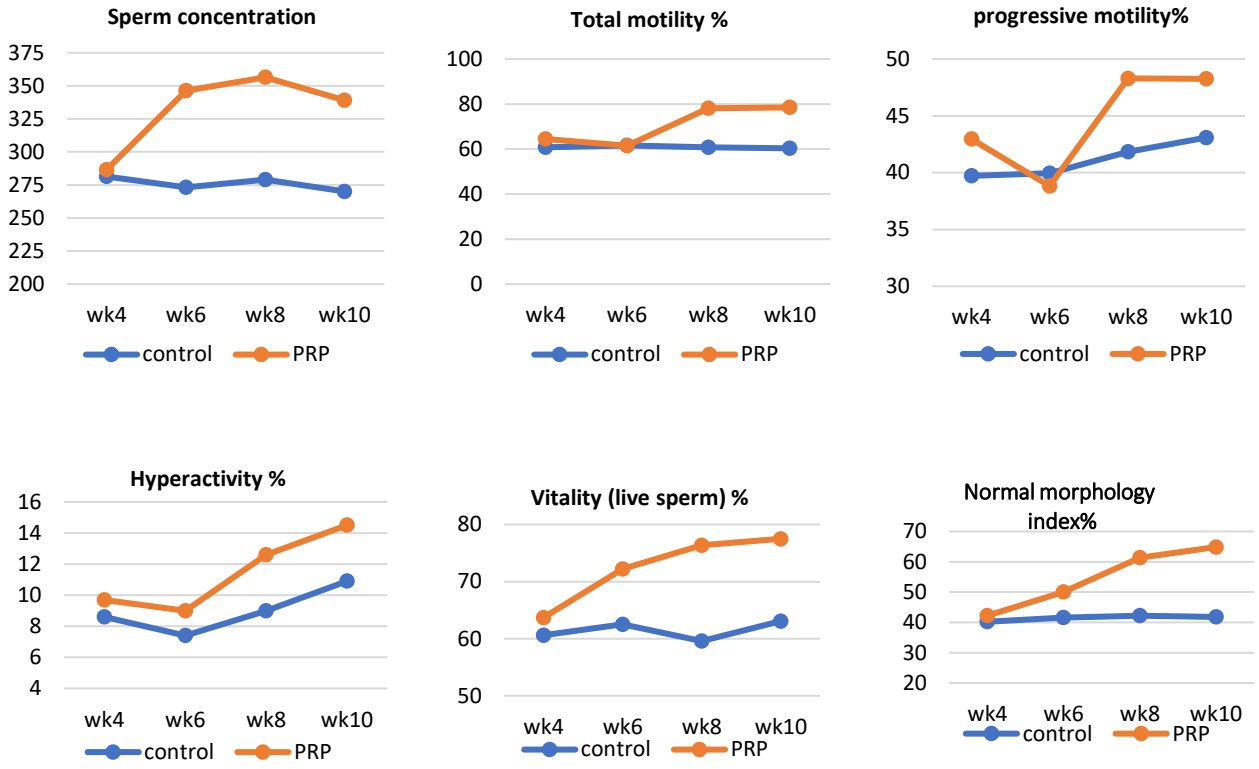
The results of the CASA analysis indicated to; the sperm concentration of PRP group at 6th, 8th and 10th weeks (346.23±37.9, 356.4± 14.11 and 339.1 ± 11.31 **(**respectively were significant (p<0.01) higher than the control group in these points (273.3± 36.21, 278.9±9.54 and 270.1 ± 9.45) respectively. Yet the sperm total motility, progressive motility, hyperactivity and vitality (live sperm) percent's of the PRP group **w**ere higher significantly (p<0.01) at the 8th wk (78.18±1.6, 48.299± 1.09, 12.6±.69 and 76.35± 2.01) respectively, compared with the control group (60.76±1.65, 41.824±1.82, 9.0±0.978 and 59.59± 1.69) respectively. Furthermore at the 10th wk were (78.50±1.58, 48.27± 48.27, 14.50±1.01 and 77.5± 1.9) respectively compared with the control group (60.34±1.41, 43.08±0.87, 10.90±0.60 and 63.08±2.17) respectively. On the other hand, the spermatozoa normal morphology index of the PRP group was significantly (p<0.01) higher than in the PRP group (50.05±1.192, 61.3±1.58 and 64.87±1.84 **(**respectively, at the 6th, 8th and 10th weeks, comparison with the control group (41.54±1.44 **,**42.19±1.54, 41.82±1.41) respectively. (Table 2 & Figure 1)

Table 2. CASA results for a comparison of sperm parameters of the control group and PRP group at four-time points of male rabbit's bucks (Mean + SEM)**.**

SP	4thwk		6thwk		8thwk		10thwk		P-value
	Control	PRP	Control	PRP	Control	PRP	Control	PRP	
SC	281.4±8.16	286.6±16.3	273.3±36.21	346.23±37.9	278.9±9.54	356.4±14.11	270.1±9.45	339.1±11.31	0.050
TM	60.77±2.17	64.54±1.62	61.63±1.4	61.49±1.53	60.76±1.65	78.18±1.6	60.34±1.41	78.50±1.58	0.000*
PM	39.72±0.91	42.97±1.23	39.94±1.37	38.82±1.61	41.824±1.82	48.299±1.09	43.08±0.87	48.27±48.27	0.000*
HA	8.60±0.72	9.70±0.96	7.40±0.78	9.0±0.26	9.0±0.978	12.6±0.69	10.90±0.60	14.50±1.01	0.000*
vitality	60.6±3.04	63.71±2.28	62.53±1.7	72.22±1.67	59.59±1.69	76.35±2.01	63.08±2.17	77.5±1.9	0.002*
NM	40.2±1.62	42.2±1.36	41.54±1.44	50.05±1.192	42.19±1.54	61.3±1.58	41.82±1.41	64.87±1.84	0.000*

PRP; Platelet-rich plasma, SEM; Standard error of the mean, SP; sperm parameters, SC; sperm concentration (sperm×10^6/ml), TM; total motility %, PM; progressive motility%, HA; hyperactivity%, vitality; (live sperm) %, NM; normal morphology index%**.*** The value of a p-value (which is less than the 0.01 significant level). This means there is a statistically significant difference between the means of the different levels of the variable time

Fig 1- CASA results (Mean) for a comparison of sperm parameters of the control group and PRP group at four-time points (4th wk, 6th wk, 8th wk and 10th wk) of male rabbit's bucks



Discussion

According to previous studies [17, 18], that were evaluated the effect of any compound on the testicular function in rabbits, should be done at least after 60-70 days, because the spermatogenesis continues from 48 to 52 days for mature spermatozoa, and 10 to 14 days are required in the epididymis [19]. In male puberty, spermatogenesis depends on testosterone action, which is necessary to initiate and maintain sperm formation, and for differentiation of the spermatids [20]. These study design was performed at four different time points (4th wk, 6th wk, 8th wk and 10th wk) to evaluate the effects of PRP intra-testicular injection on testosterone, VEGF and IGF-1 levels in the seminal plasma of male rabbits. The testosterone had a high level (P<0.01) in the 4th and 6th weeks (Table 1), this finding agrees with previous studies, which concluded that PRP injection stimulates Leydig cells to increase testosterone production [21, 22]. During spermatogenesis, the VEGF can affect meiosis and cell growth by stimulating the formation of new blood vessels, which help to deliver vitamins and minerals to the testicular parenchyma and allow it to produce mature spermatozoa [23, 24]. The our study results, indicate the PRP intra-testicular injection increased (P<0.01) the VEGF concentration in seminal plasma at the 4th and 6th weeks (Table 1), these findings are in agreement with previous studies, which showed that VEGF of PRP supports germ cell proliferation, lifecycle maintenance and inhibition of apoptosis [25, 26]. Furthermore, the results of this study indicate that intra-testicular injection of PRP may increase the level of IGF-1 in seminal plasma at 4 weeks (p<0.05), these results correlated with improved sperm parameters, which is probably through that a biological role of IGF-1 by reducing the apoptosis of germ cells and increasing density according to information by [27, 28] that were referred to the testis development depended on the activation of both insulin and IGF1. Yet, these

results disagree with study of [29, 30] which was indicated to PRP injection can inhibit IGF-1 synthesis in the testes due to negative feedback on the hypothalamus, which lowers growth hormone-releasing hormone (GHRH) levels, this will subsequently impact the anterior pituitary's ability to produce the growth hormone.

The main objective of this project is to evaluate the effects of PRP intra-testicular injection on sperm parameters. Our finding revealed that the spermatozoa concentration significantly ($P<0.01$) increased in the PRP group in the 8th week. Furthermore, the high percentage of total motility, progressive motility, vitality, hyperactivity and normal morphology referred to the positive effect of PRP therapy in the 8th and 10th weeks (Table 2 & Figure 1). These results were in agreement with [12] that were indicated the PRP growth factors may increase the blood supply to the parenchyma and stimulate the testicular cells to produce good quality and quantity of spermatozoa. However, the PRP injection has a positive effect on the testes function of rats with induced Diabetes Mellitus [31]. Moreover, PRP therapy can enhance the viability and motility, which affected by oxidative stress in human spermatozoa [32].

The PRP has a protective effect on testicular tissues, which suffer from Ischemia/ Reperfusion Injury in rats by increasing antioxidant defense and inhibiting neutrophil infiltration and oxidative stress [33]. The PRP intra-testicular injection from 6th to 10th weeks can increase the normal spermatozoa index by a significant difference statistic ($P<0.01$) vs. the control group, through the growth factor which provides a high nutritious supply to the testes cells, which leads to improving the spermatogenesis process to produce good spermatozoa cells (Table 2& Figure 1). The results of [34, 35]. In addition, the conclusion of [36, 37] One of the most important results that we obtained in this study is that the PRP has many properties, which may serve potential effects in spermatozoa regeneration in healthy rabbits by evaluating its impact on testosterone, VEGF, IGF1 in seminal plasma and sperm parameters. More samples are required for additional investment, due to limitations; we were only able to experiment with a small sample size in this study. [38, 39].

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Conclusions

The findings of this study revealed that intra-testicular injection of PRP significantly improves testicular function and spermatozoa normal morphology when compared to the control group at 8th and 10th weeks. These results confirmed by measuring testosterone, VEGF, and IGF-1 levels in seminal plasma, which were exceptionally high, particularly at the 4th and 6th weeks. However, more research is required to understand the connection between these processes. Researchers hope to conduct larger, multicenter randomized control trials in the future.

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Disclosure statement

There is no any conflict of interest.

References

1. Yániz JL, Silvestre MA, Santolaria P, Soler C. CASA-Mot in mammals: an update. Reproduction, Fertility and Development. 2018;30(6):799-809. [PubMed]
2. Zhu Y, Yuan M, Meng HY, et al. Basic science and clinical application of platelet-rich plasma for cartilage defects and osteoarthritis: a review. Osteoarthritis and cartilage. 2013;21(11):1627-1637. [PubMed]

3. Sariguney Y, Yavuzer R, Elmas C, Yenicesu I, Bolay H, Atabay K. Effect of platelet-rich plasma on peripheral nerve regeneration. Journal of reconstructive microsurgery. 2008;24(03):159-167. [PubMed]
4. Prahalad P, Ding VY, Zaharieva DP, et al. Teamwork, Targets, Technology, and Tight Control in Newly Diagnosed Type 1 Diabetes: Pilot 4T Study. The Journal of Clinical Endocrinology & Metabolism. Published online 2021. [PubMed]
5. Belk JW, Kraeutler MJ, Houck DA, Goodrich JA, Dragoo JL, McCarty EC. Platelet-rich plasma versus hyaluronic acid for knee osteoarthritis: a systematic review and meta-analysis of randomized controlled trials. The American journal of sports medicine. 2021;49(1):249-260. [Google Scholar]
6. Bos-Mikich A, de Oliveira R, Frantz N. Platelet-rich plasma therapy and reproductive medicine. Journal of assisted reproduction and genetics. 2018;35(5):753-756. [PubMed]
7. Dawood AS, Salem HA. Current clinical applications of platelet-rich plasma in various gynecological disorders: An appraisal of theory and practice. Clinical and experimental reproductive medicine. 2018;45(2):67. [PubMed]
8. Dehghani F, Sotoude N, Bordbar H, Panjeshahin MR, Karbalay-Doust S. The use of platelet-rich plasma (PRP) to improve structural impairment of rat testis induced by busulfan. Platelets. 2019;30(4):513-520. [PubMed]
9. Sekerci CA, Tanidir Y, Sener TE, et al. Effects of platelet-rich plasma against experimental ischemia/reperfusion injury in rat testis. Journal of pediatric urology. 2017;13(3):317-e1. [PubMed]
10. Kutluhan MA, Özsoy E, Şahin A, et al. Effects of platelet-rich plasma on spermatogenesis and hormone production in an experimental testicular torsion model. Andrology. 2021;9(1):407-413. doi:10.1111/andr.12895.
11. Al-Nasser R. The Effectiveness of Autologous Platelet-Rich Plasma (PRP) in the Therapy of Infertile Men with Non-Abstractive Azoospermia. Journal of Reproductive Medicine, Gynaecology & Obstetrics. 2018;3(1):1-4. doi:10.24966/rmgo-2574/100011
12. Lai F, Kakudo N, Morimoto N, et al. Platelet-rich plasma enhances the proliferation of human adipose stem cells through multiple signaling pathways. Stem cell research & therapy. 2018;9(1):107. [Google Scholar]
13. Al-Bayati MA, Khamas W, Al Ameria B. The Importance to Implement and Enforce of Standardized Guidelines For The Care And Use Of Laboratory Animals in Research And Teaching In Iraqi Scientific Institutions. TOFIQ Journal of Medical Science. 2015;2:34-43. [Google Scholar]
14. Kathiravan P, Kalatharan J, Karthikeya G, Rengarajan K, Kadirvel G. Objective sperm motion analysis to assess dairy bull fertility using computer-aided system–a review. Reproduction in Domestic Animals. 2011;46(1):165-172. [PubMed]
15. Ariagno JI, Mendeluk GR, Furlan MJ, et al. Computer-aided sperm analysis: a useful tool to evaluate patient's response to varicocelectomy. Asian journal of andrology. 2017;19(4):449. [PubMed]
16. Duncan DB. Multiple range and multiple F tests. Biometrics. 1955;11(1):1-42.
17. Amann RP. Use of animal models for detecting specific alterations in reproduction. Fundamental and Applied Toxicology. 1982;2(1):13-26. [Google Scholar]
18. Morton D. The use of rabbits in male reproductive toxicology. Environmental Health Perspectives. 1988;77(6):5-9. doi:10.1289/ehp.88775 [Google Scholar]
19. Castro ACS, Berndtson WE, Cardoso FM. Plasma and testicular testosterone levels, volume density and number of

Leydig cells and spermatogenic efficiency of rabbits. Brazilian Journal of Medical and Biological Research. 2002;35(4):493-498. [PubMed]
20. Omotuyi IO, Ologundudu A, Onwubiko VO, Wogu MD, Obi FO. Hibiscus sabdariffa linn. Anthocyanins alter circulating reproductive hormones in rabbits (oryctolagus cuniculus). Journal of Diabetes and Endocrinology. 2010;1(3):36-45. [Google Scholar]
21. Zirkin BR, Papadopoulos V. Leydig cells: formation, function, and regulation. Biology of reproduction. 2018;99(1):101-111. [PubMed]
22. Walker WH. Non-classical actions of testosterone and spermatogenesis. Philosophical Transactions of the Royal Society B: Biological Sciences. 2010;365(1546):1557-1569. [PubMed]
23. Salamanna F, Veronesi F, Maglio M, Della Bella E, Sartori M, Fini M. New and emerging strategies in platelet-rich plasma application in musculoskeletal regenerative procedures: general overview on still open questions and outlook. BioMed Research International. 2015;2015. [PubMed]
24. Iyibozkurt AC, Balcik P, Bulgurcuoglu S, Arslan BK, Attar R, Attar E. Effect of vascular endothelial growth factor on sperm motility and survival. Reproductive BioMedicine Online. 2009;19(6):784-788. doi:10.1016/j.rbmo.2009.09.019 [PubMed]
25. Griffeth RJ, Bianda V, Nef S. The emerging role of insulin-like growth factors in testis development and function. Basic and clinical andrology. 2014;24(1):1-10. [PubMed]
26. Caires KC, de Avila J, McLean DJ. Vascular endothelial growth factor regulates germ cell survival during establishment of spermatogenesis in the bovine testis. Reproduction. 2009;138(4):667. [PubMed]
27. Yao J, Zuo H, Gao J, Wang M, Wang D, Li X. The effects of IGF-1 on mouse spermatogenesis using an organ culture method. Biochemical and biophysical research communications. 2017;491(3):840-847. [PubMed]
28. Dupont J, Holzenberger M. Biology of insulin-like growth factors in development. Birth Defects Research Part C: Embryo Today: Reviews. 2003;69(4):257-271. [PubMed]
29. Barrett EJ. Endocrine regulation of growth and body mass. Medical Physiology 3rd ed Elsevier, Philadelphia. Published online 2017:990-1005. [Google Scholar]
30. Bakacak M, Bostanci MS, İnanc F, et al. Protective effect of platelet rich plasma on experimental ischemia/reperfusion injury in rat ovary. Gynecologic and obstetric investigation. 2016;81(3):225-231. [Google Scholar]
31. Rizal DM, Hermilasari RD, Wirohadidjojo YW. Effect of Platelet-Rich Plasma (PRP) on testicular damage in streptozotocin-induced diabetic rats. Bali Medical Journal. 2020;9(1):351-355. doi:10.15562/bmj.v9i1.1718
32. Bader R, Ibrahim JN, Moussa M, et al. In vitro effect of autologous platelet-rich plasma on H2O2-induced oxidative stress in human spermatozoa. Andrology. 2020;8(1):191-200. [PubMed]
33. Gazia MA. Histological Study on the Possible Ameliorating Effect of Platelet Rich Plasma on Ischemia/Reperfusion Injury in Testicular Torsion Model in Adult Albino Rat. Egyptian Journal of Histology. 2020;43(2):614-629. [Google Scholar]
34. Bir SC, Esaki J, Marui A, et al. Angiogenic properties of sustained release platelet-rich plasma: characterization in-vitro and in the ischemic hind limb of the mouse. Journal of vascular surgery. 2009;50(4):870-879. [PubMed]
35. Sánchez-González DJ, Méndez-Bolaina E, Trejo-Bahena NI. Platelet-rich plasma peptides: Key for regeneration. International Journal of Peptides.

2012;2012. doi:10.1155/2012/532519
36. Abo-Elmaksoud A, Sinowatz F. Expression and localization of growth factors and their receptors in the mammalian testis. Part I: Fibroblast growth factors and insulin-like growth factors. Anatomia, histologia, embryologia. 2005;34(5):319-334. [Google Scholar]
37. He J, Dong C, You R, Zhu Z, Lv L, Smith GW. Localization of epidermal growth factor (EGF) and its receptor (EGFR) during postnatal testis development in the alpaca (Lama pacos). Animal reproduction science. 2009;116(1-2):155-161. [Google Scholar]
38. Abdel-Naser MB, Wollina U, Lohan M, Zouboulis CC, Altenburg A. Schistosomiasis (bilharziasis) ova: an incidental finding in testicular tissue of an obstructive azoospermic man. Andrologia. 2018;50(10):e13131. [PubMed]
39. Abdel-Naser MB, Altenburg A, Zouboulis CC, Wollina U. Schistosomiasis (bilharziasis) and male infertility. Andrologia. 2019;51(1):e13165. [Google Scholar]