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Effect of the incorporation of plasma rich of platelets on the spermatozoa physiology of ram semen

Abstract

Plasma rich in platelets (PRP) have been used for healing processes, maxillary oral surgery, plastic surgery, although its use in andrology is null; however, there is no information about its effect on sperm motility and morphology. The aim of this study was to compare the effect PRP in inclusion ram semen in the parameters motility (Mt) and morphometrics (Mp). Semen samples (n=8) were collected by artificial vagina from two rams located in the North of Santander, Colombia (L 7°54′N, L 72°30′W). Obtain the blood sample by venous puncture of the jugular the tubes (Citrated and EDTA) at 3000 g/3 min centrifuged, after separation PRP (rich and poor). After the initial evaluation, Eppendorf tubes of 2mL, 500µL, saline solution, 10µL of fresh semen and 10µL PRP (rich or poor) were placed 5 min after preparation of the samples, (F:fresh, N: saline solution, CPr: citrated platelets rich; CPp: citrated platelets poor; EPr: EDTA platelets rich; EPp EDTA platelets poor). sperm was analyzed per software the Sperm Class Analyser (ISAS, Proiser, Spain) were calculated for Mp and for Mt, parameters were compared between treatments (T) by ANOVA. Significant differences (P<0.001) were found between T for Mp and Mt. According to these results, the sperm head dimensions (SHD) of semen samples with inclusion EPr were bigger and the velocity was higher. An increase in SHD has been previously described due to osmolar adjustment procedures, the same happened with the parameter of Mt and linearity, improving the characteristic up to 6% with respect to fresh semen. it is observed that the PRP improves the Mp and the Mt of the ram spermatozoa, thanks to the growth factors and the components of its dense granules

Keywords: ovine, CASA, plasma, platelets, EDTA, citrate

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Introduction

Platelets are derivatives of megakaryocytes, have α granules that contain many secretory proteins, belong to the family of growth factors, cytokines and chemokines, which strongly influence the healing of wounds (PDGF, IGF-I, IGF-II, TGF, EGF, FGF) and dense granules (serotonin, ATP, calcium), are involved in acceleration and modulation in the healing processes through factors growth (GF).[1] The plasma Rich platelets (PRP) is a concentrated fraction of platelets higher than basal levels, researchers such as Anitua et al,[2] and Rodríguez et al.[3] They suggested that the PRP should reach a platelet concentration of 3 to 5 times higher than the normal level, when the blood is centrifuged with anticoagulated, the upper layer is formed three layers (density 1.03), composed of plasma, the middle layer (density 1.06), composed of white blood cells and platelets and the lower layer layer (density 1.09), composed of red blood cells.

PRP is an important part of the group of cells found in blood plasma, studied and used in various medical specialties including dentistry, orthopedics, neurosurgery, ophthalmology, maxillofacial surgery, and cosmetic surgery. In veterinary medicine, work has been carried out mainly in horses and companion animals with ligament injuries, tendons, and osteoarthritis, ophthalmic surgeries, among others, but until now there have been no reports of their use in animal reproduction in male and less in the protection of animals. Sperm cells, with this study, intends to evaluate In Vitro its applicability in ram semen.

Material and method

Animals and semen collection

The study in the ranch Siete Colores, production unit sheep. Semen was obtained from two mature Katahdin Ram (2- years-old), located in the municipality of Los Patios (Norte de Santander. Colombia) in an agro-ecologically characterized area as tropical dry forest. Semen was collected from each animal one a week, using an artificial vagina and estrous females as mounts for the Ram.

Research protocol

After collection, semen samples were evaluated in fresh at a ratio of 1:200 with software ISAS® (Proiser, Spain) was assessed objectively using the motility module. Sperm concentration was measured with a digital photometer (DVM, Rapid TEST II, Sperm Concentration Analyzer®, MAI, USA). The percentage of spermatozoa with normal morphology and intact acrosomal membranes was assessed by visual examination of microscopic slides stained with Diff-Quik (DQ) (Baxter DADE AG 3186, Düdingen, Switzerland).

Fort, he evaluated motility and morphometric the spermatozoa were captured randomly in different fields in the module morphometric, this

process was performed manually by an interactive selection of cells to avoid the inclusion of foreign particles that interfered in the way of the subsequent image processing. To the animals after the seminal collection, the neck area was washed with alcohol to obtain the blood sample by venous puncture of the jugular, by means of a vacutainer® (Becton, Dickinson, and company) caliber 18, was taken the plastic cap (shirt) placing the needle, with the gloved hand the vein is vented for the taking of the same, the tubes (citrated and EDTA) are filled approximately 4 mL, 2 aliquots of 2mL were taken in Eppendorf tubes (2mL), taken to a centrifuge Thermo scientific Spreso® (Shanghai, China) at 3000 g for 3 minutes, after separation the poor PRP and the rich one were obtained depending on the tube of the sample taken (citrated or with EDTA). After the initial evaluation, Eppendorf tubes of 2 mL, 500µL, and saline solution, 10µL of fresh semen and 10µL of autologous platelet concentrate (rich or poor) were placed 5 minutes after preparation of the samples (Table 1).

Table 1 Treatment protocol

Tube	PRP	Treatment
		Fresh
		NaCl
Citrated	Poor	CPp
	Rich	CPr
EDTA	Poor	Epp
	Rich	EPr

For morphometry parameters, a total of eight morphometric parameters were assessed. Of these, four parameters related to head size: length (L in mm, along the main axis), width (W in mm, along the smaller axis), area (A in mm^2, the total area of the sperm head) and perimeter (P in mm, as the sum of external boundaries); The measurements of each individual sperm cell were saved in an Excel (Microsoft Corporation, Redmond, Washington, USA) compatible database by the software for further analysis. Parameters of the analysis software were set according to Dorado et al.[4] Briefly, spermatozoa with mean average path velocity (VAP) ≤10 mm s^{-1} were considered immobile. Spermatozoa with a VAP≥90 mm s^{-1} were considered rapid, and spermatozoa deviating ≤25% from a straight line were designated as linear motile. The following kinetic traits were assessed: curvilinear velocity (VCL), the total distance travelled by the sperm head per unit time; straight line velocity (VSL), the net distance gain of the sperm head per unit time; VAP, the length of a derived 'average' path of sperm head movement per unit time; wobble (WOB), calculated as (VAP/ VCL)*100; linearity (LIN), calculated as (VSL/VCL)*100; straightness (STR), calculated as (VSL/VAP)*100; beat cross frequency (BCF), the number of times the curvilinear path crosses the average path per unit time; approximation of the flagellar beat frequency for seminal sperm (in Hz); and amplitude of lateral head displacement (ALH), the width of the head movement envelope.

Result s and discussion

The multiple variations of osmolarity and oxidation determine changes in the structure of the plasma membrane, making it a mosaic of gel and fluids in different phases, generating an intramembranous barrier that prevents ion-free diffusion. In addition to mobility, several parameters of sperm morphology are affected by oxidation, such as the increase in the percentage of reacted sperm.[5] Alcalaz[6] explains that the three-speed variables that are determined through CASA are the main kinematic parameters related to fertilization. In the same way, authors like Holt et al,[7] in pigs; Silva & Gadella[8] in dogs, showed that high speeds are points of reference for *In Vivo* and *In Vitro* fertilization. Dorado et al,[9] in goats concluded that the velocity parameters (VCL, VAP) are predictors of the resistance potential to the freezing process. However, Gillan et al,[10] defined that for bulls the VSL is the parameter that is related to fertilization (Table 2). The discrepancies between treatments (NaCl, CpC) for CASA sperm motility variables are due to the sensitivity of sperm to osmotic shocks.[11,12] Zhu et al,[13] and Blesbois,[14] describe that in the spermatic cells there is a marked deterioration in the plasma and acrosomal membrane; Authors such as Fraczek & Kurpisz[15] describe damage at the level of DNA defragmentation and mitochondrial damage. Understanding the decrease of individual mobility when the semen is evaluated after the collection time has passed.

Table 2 Mean and standard error of the descriptors of sperm morphometry according to treatment performed on the sheep evaluated

	Treatment					
	Fresh	**NaCl**	**CPp**	**CPr**	**Epp**	**EPr**
Large	9.44±0.06^{c}	9.40±0.01^{c}	9.74±0.03^{b}	9.75±0.05^{b}	9.83±0.12^{a}	9.94±0.08^{a}
Width	5.34±0.03^{c}	5.04±0.005^{c}	5. 57±0.01^{b}	5.56±0.05^{b}	5.63±0.05^{a}	5.67±0.06^{a}
Área	43.84±0.46^{c}	39.66±0.07^{d}	45.75±0.63^{b}	46.74±0.31^{b}	46.91±0.79^{b}	48.64±0.36^{a}
perimeter	26.38±0.14^{cd}	25.35±0.04^{d}	26.98±0.04^{c}	27.48±0.13^{bc}	27.53±0.27^{b}	28.63±0.18^{a}

The results are expressed in mean ± standard error for the analyzed semen samples of the sheep. Equal letters (a, a) do not show significant differences (P≥0.05), different letters (a, b) show significant differences (P≤0.001)

In the investigation, the spermatic velocities in the different treatments showed significant differences between the processes (P≤0,01) VCL, VSL, VAP, LIN, STR, WOB, ALH, BCF. Because sperm survival, mobility, and fertility decrease after the collection process; The characteristics of fresh semen (F) often correlate poorly with male quality.[16] In contrast, a correlation between certain variables of mobility, abnormalities, and integrity of the acrosome can predict fertilizing capacity after cryopreservation.[9] While Evans et al,[17] and Leboeuf et al,[18] have proposed that sperm morphology is a criterion for the selection of fertile ejaculates. Ejaculated with a low percentage of morphological abnormalities in F, they have, on average, the best values of mobility and speed if they are evaluated after thawing.[19–22] However, Muiño-Otero et al,[23] concluded that the fastest and most progressive sperm resist the damaging effect of cryopreservation better (Table 3).

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Table 3 Mean and standard error of the descriptors of sperm motility according to treatment performed on the sheep evaluated

	Treatment					
	Fresh	**NaCl**	**CPp**	**CPr**	**EPp**	**EPr**
VCL	118.04±1.39^{b}	112.92±1.44^{c}	106.50±0.93^{d}	113.56±1.26^{bc}	113.62±1.12^{bc}	125.34±1.18^{a}
VSL	65.97±1.30^{b}	58.97±1.19^{cd}	50.97±0.71^{d}	59.92±1.03^{bc}	62.98±0.98^{bc}	71.62±1.12^{a}
VAP	74.10±1.17^{b}	71.12±1.18^{b}	65.57±0.71^{c}	74.16±1.01^{b}	74.17±0.95^{b}	83.15±1.04^{a}
LIN	53.46±0.07^{b}	50.48±0.06^{c}	47.21±0.04^{d}	51.52±0.05^{bc}	52.99±0.05^{b}	55.76±0.06^{a}
STR	82.33±0.06^{a}	78.25±0.06^{cd}	74.13±0.04^{e}	76.82±0.05^{de}	79.79±0.04^{bc}	81.34±0.05^{ab}
WOB	61.19±0.05^{c}	61.33±0.05^{c}	60.66±0.03^{c}	63.70±0.04^{ab}	63.06±0.04^{b}	64.89±0.04^{a}
ALH	3.95±0.04^{a}	3.98±0.04^{a}	3.91±0.03^{a}	3.88±0.04^{b}	3.88±0.03^{c}	4.01±0.01^{a}
BCF	7.47±0.10^{a}	6.58±0.09^{b}	5.84±0.05^{d}	6.26±0.06^{c}	6.49±0.07^{bc}	7.29±0.08^{a}

VCL, Curvilinear velocity (µm / s); VSL, rectilinear velocity (µm / s); VAP, average velocity (µm/s); LIN, linearity index (%); STR, straightness index (%); WOB, oscillation index (%); ALH, lateral displacement of the head; (µm) BCF, Frequency of the sperm head beat (Hz); (a, b, c), different letters in the rows denote significant statistical differences (P <0.001)

Sperm motility depends on endogenous and exogenous factors. According to Vera-Muñoz et al,[24] the appearance of this motility is associated with an intracellular increase of cyclic AMP and protein-dependent kinases of cyclic AMP and the decrease of Ca^{++} and calmodulin. Progressive mobility is associated with an increase in intracellular carnitine in sperm. The research suggests that the use of PRP containing serotonin Metcalf et al,[25] induces the increase in the VCL values, achieving values similar to the study by Jiménez-Trejo et al,[26] in human semen, which used serotonin in high concentrations, improving VCL, while in low serotonin concentrations VSL increases as VAP but not VCL. (VCL: includes only the displacement of the head of the sperm but not the speed of movement of the main piece, as do VSL and VAP). Rubio-Guillen et al,[27] determines that the factors that influence sperm motility are not exactly known, but it is known that they are of epididymal origin and that they could trigger protein phosphorylations dependent on cyclic AMP, therefore, also of the adenylate system cyclase. These epididymal secretions contain, in the case of the rams, a 66kD protein dependent on androgens and involved in the acquisition of the binding capacity with ZP3 Dacheux & Dacheux[28] since 1951, the consensus is called sperm capacitation, taking into account that during training, proteins within the membrane of the sperm migrate, thanks to the loss of intramembrane cholesterol and proteins can move, thus forming domains with or without the latter. In areas where there are no proteins, the MP and the outer acrosomal membrane will fuse. This fusion begins the process of exocytosis of the acrosomal content.[29] Moura & Memili[30] determine that the angiotensin converted enzyme (ACE) is a seminal component related to the kallikrein system.[31] ACE catalyzes the formation of angiotensin II and protects sperm receptors, intensifying motility-related events.[32] In the morrug, the activity of the ACE in the seminal plasma has a positive correlation with sperm concentration and fertility.[33,34] In bovine seminal plasma ACE inhibits the decreasing number of sperm with progressive motility and inhibits the acrosomal reaction after in vitro training.[35]

According to Miki[36] there is growing evidence that ATP supports sperm motility is generated by glycolysis, which takes place throughout the main piece.[37] It has been shown that the frequency of flagella beating is proportional to the rate of ATP hydrolysis by dynein when the wave remains constant.[38] Serotonin improves glycolytic flux through the activation of 6-phosphofructose-1-kinase (PFK), which is produced through the modulation of the binding of the enzyme to the membrane cytoskeleton.[39] In addition, the activity of PFK correlates with a complete glycolytic pathway in the muscle.[40] Serotonin increases glucose consumption in skeletal muscle and suggests that this neurohormone can regulate the metabolism of cellular energy;[41] therefore, in sperm, it is likely that serotonin plays a similar role. In the study by Jiménez-Trejo[26] by applying high levels of serotonin-induced rapid sperm head movements, while at lower concentrations the linearity of sperm displacement increased, because serotonin increased the level of tyrosine phosphorylation, indicating that this indoleamine it induces hyperphosphorylation of the dynein, in the intermediate piece, which results in a non-physiological displacement of the sperm.[42,43] However, the effects of serotonin on the different parameters of sperm motility, at different concentrations, suggest that it can participate modulating the displacement of sperm at different molecular levels, but this aspect requires further investigation. Because it is contrasted with the investigations of Safarinejad.[44]

Researchers like Hernández & Chirinos.[45] They explain that the endometrial cells of the uterus also secrete interleukin-6 (IL-6), which is a mediator of the inflammatory response that increases during the periovulatory period.[46] IL-6 induces sperm capacitation by increasing the phosphorylation of the tyrosine protein and enhances the spontaneous ionophore-induced by calcium. In addition, recent research indicates a broad presence of fibroblast growth factor 2 (FGF2) in the uterus of the mouse and the oviduct, this protein is capable of increasing sperm motility, intracellular calcium levels and in vitro acrosomal loss.[47] The differences in the size of the head between the sperm in the different treatments occur because the sperm has modified the function of the membrane and this sperm will have lost or gained part of their intracellular content as a result of the osmotic change.[48] This explains the excessive growth of the sperm cells in the face of physical-chemical changes and the physiological adaptation of the cells to the different stressors of the components that add them in each process. It should be noted that the treatment of EPp and EPr have higher values than those taken in the fresh sample, this can be justified in the role that the biopharmaceutical performs when included in sheep semen. Progressive rehydration of sperm occurs due to changes in osmolarity and temperature, sometimes it can cause

acrosomal damage, this change in the distribution of enzymes in the membranes or differences in the structure of DNA can be induced in free radicals.[49,50] However, the functionality parameters of the membranes and DNA integrity showed no changes in the normal values.

Authors such as Tuncer et al,[51] conclude that the possible physiological reaction of sperm cells to the change in morphology can be attributed to oxidative stress (of the medium), or to the effects of seminal plasma, which protects the sperm in the process of manipulation. When comparing the data obtained in the research with those presented for ovines in the tropics, no significant differences were observed as reported by Rubio-Guillen,[27] but if they are below the data presented by Sepúlveda et al,[52] this difference can be attributed to many factors, such as the fixation technique used, the staining procedure, the individual variation or the ASMA system used; in this sense, the exact interpretation of the results should be different when using nuclear or cellular stains.[53–59]

Conclusions

When comparing the descriptors of mobility with the different treatments, the damage that was caused from the sample taking, to the addition of NaCl and the other inclusions of autologous platelet concentrates was evidenced, starting from the mobility that is the first parameter that was affected by the deleterious effect of the physiological process of formation of free radicals of oxygens and waste metabolites of sperm cells, observing a gradual decrease in the speed and linearity of sperm. Except in the EPr treatment centrifuged with EDTA. During the investigation, the addiction of autologous platelet concentrate rich centrifuged with EDTA (EPr), improved sperm motility with respect to fresh semen. This process seems to be favored by the action of the fibroblastic growth factor that induces sperm motility, interleukin 6 (IL6) that induces sperm capacitation through calcium ionophore induction pathways and the increase of tyrosine phosphatase protein. When comparing the descriptors of sperm morphometry, the change of size and shape values in the different treatments explains the change of the spermatic membranes, the injury of the passage from one medium to another and the time of adaptation to the medium, the temperature on the membranes and the internal fluids of the sperm cell in sheep. It can be concluded that adding plasma Rich of platelets as a biopharmaceutical is highly effective in the selection of fast sperm, being cheaper and easier than concentration gradients.

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Conflicts of interest

Author declares that there are no conflicts of interest.

References

1. Carmona J, Arguelles D, Prades M. Transforming growth factor beta-3 and nitric oxide levels in four autologous platelet concentrates and plasma derived from equine blood. *Arch Med Vet*. 2008;40:155–160.
2. Anitua E, Andia I, Ardanza B, et al. Autologous platelets as a source of proteins for healing and tissue regeneration. *Thromb Haemost*. 2004;91(1):4–15.
3. Rodríguez J, Palomar M, García J. Platelet-rich plasma: biological foundations and applications in maxillofacial surgery and facial aesthetics / Platelet-rich plasma: biology and applications in maxillofacial surgery and facial aesthetics. *Rev Esp Cir Oral Maxilofac*. 2012;34(1):8–17.
4. Dorado J, Morales R, Molina A, et al. Effect of inbreeding depression on bull sperm quality and field fertility. *Reprod Fertil Dev*. 2017;29(4):712–720.
5. Valcárcel A, De las Heras M, Pérez L, et al. Fluorescent staining as a method of assessing membrane damage and post-thaw survival of ram spermatozoa. *Theriogenology*. 1994;4:483–489.
6. Alcalaz L. *Centrifugación coloidal del semen crioconservado de perro para la selección de espermatozoides*. España: Tesis Doctoral, Universidad de Córdoba; 2013. p. 57.
7. Holt C, Holt W, More H, et al. Objectively measured sperm motility parameters correlate with the outcomes of on-farm inseminations: results of two fertility trials. *J Androl*. 1997;18:312–323.
8. Silva P, Gadella B. Detection of damage in mammalian sperm cells. *Theriogenology*. 2006;65(5):958–978.
9. Dorado J, Hidalgo M, Muñoz A, et al. Assessment of goat semen freezability according to the spermatozoa characteristics from fresh and frozen samples. *Animal Reproduction Science*. 2009;112:150–157.
10. Gillan L, Evans G, Maxwell W. Flow cytometric evaluation of sperm parameters in relation to fertility potential. *Theriogenology*. 2005;63(2):445–457.
11. Watson P. The causes of reduced fertility with cryopreserved semen. *Anim Reprod Sci*. 2000;60(61):481–492.
12. Osorio C. *Valoración computarizada de la integridad funcional de la membrana plasmática, movilidad y morfología espermática en semen criopreservado de Búfalo*. Tesis maestría: Universidad del Zulia; 2013. p. 106.
13. Zhu W, Liu X. Cryodamage to plasma membrane integrity in head and tail regions of human sperm. *Asian J Androl*. 2000;2(2):135–138.
14. Blesbois E. Current status in avian semen cryopreservation. *World's Poult Sci J*. 2007;63(2):213–222.
15. Fraczek M, Kurpisz M. The redox system in human semen and peroxidative damage of spermatozoa. *Postepy Hig Med Dosw*. 2005;59:523–534.
16. Verstegen J, Iguer-Ouada M, Onclin K. Computer assisted semen analyzer in andrology research and veterinary practice. Theriogenology. 2002;57(1):149–179.
17. Evans G, Maxwell W. *Salmon's Artificial*. Insemination of Sheep and Goats. London: Butterworth; 1990. p. 107–141.
18. Leboeuf B, Restall B, Salamon S. Production and storage of goat semen for artificial insemination. *Animal Reproduction Science*. 2000;62(1):113–141.
19. Hidalgo M, Rodríguez I, Dorado J. The effect of cryopreservation on sperm head morphometry in Florida male goat related to sperm freezability. *Animal Reproduction Science*. 2007;100:61–72.
20. Vásquez J, Núñez V, Florentini E, et al. Effects of five cryoprotective agents on quality of sheep epididymal spermatozoa during pre-freezing. *Livestock Science*. 2013;152(1):94–99.
21. Rahmatzadeh M, Kohram H, Zare Shahneh A, et al. Antioxidative Effect of BHA in Soya Bean Lecithin-Based Extender Containing Glycerol or DMSO on Freezing Capacity of Goat Semen. *Reprod Domest Anim*. 2007;52(6):1–7.
22. Ledesma A, Zalazar L, Fernández-Alegre E, et al. Seminal Plasma Proteins Modify the Distribution of Sperm Subpopulations in Cryopreserved Semen of Rams With Lesser Fertility. *Anim Reprod Sci*. 2017;184:44–50.

23. Muiño-Otero R, Fernández M, Peña A. Post-thaw survival and longevity of bull spermatozoa frozen with an egg yolk-based or two egg yolk-free extenders after an equilibration period of 18h. *Reprod Domest Anim*. 2007;42(3):305–311.

24. Vera-Muñoz O. Physiology of bovine sperm. In: Sustainable development of dual purpose livestock. C Gonzalez-Stagnaro, E Soto-Belloso, editors. *Girarz Foundation*. 2008;5(40):495–504.

25. Metcalf K, Mandelbaum B, Mcllwraith W. Application of Platelet-Rich Plasma to Disorders of the Knee Joint. *Cartilage*. 2013;4(4):295– 312.

26. Jiménez Trejo F, Tapia-Rodríguez M, Cerbón M, et al. Evidence of 5-HT components in human sperm: implications for protein tyrosine phosphorylation and the physiology of motility. *Reproduction*. 2012;144(6):677–685.

27. Ubio-Guillen J. *Effect of cryopreservation on the structural and functional integrity of sheep sperm under tropical conditions*. Venezuela: University of Zulia; 2017. p. 153.

28. Dacheux F, Dacheux J. *The epididymis and the annex glands*. In: Reproduction in mammals and humans. Coordinators: Charles Thibault and Marieclaire Levasseur. France: Ellipses Marketing Edition; 2001;14:455-468.

29. Kraus M, Tichá M, Zelez B. Characterization of human seminal plasma proteins homologous to boar AQN spermadhesins. *J Reprod Immunol*. 2005;65(1):33–46

30. Moura A, Memili E. Functional aspects of seminal plasma and sperm proteins and their potential as molecular markers of fertility. *Anim Reprod*. 2016;13(3):191–199.

31. Hohlbrugger G, Pschorr J, Dahlheim H. Angiotensin I-converting enzyme in the ejaculate of fertile and infertile men. *Fertil Steril*. 1984;41:324–325.

32. Vinson G, Mehta J, Evans S, et al. Angiotensin II stimulates sperm motility. *Regul Pept*. 1996;67(2):131–135.

33. Métayer S, Dacheux F, Guérin Y, et al. Physiological and enzymatic properties of the ram epididymal soluble form of germinal angiotensin I-converting enzyme. *Biol Reprod*. 2001;65(5):1332–1339.

34. Gatti J, Castella S, Dacheux F, et al. Post-testicular sperm environment and fertility. *Anim Reprod Sci*. 2004;82(83):321–339.

35. Costa D, Thundathil J. Characterization and activity of angiotensin-converting enzyme in Holstein semen. *Anim Reprod Sci*. 2012;133(1):35–42.

36. Miki K. Energy metabolism and sperm function. *Society of Reproduction and Fertility Supplement*. 2007;65:309–325.

37. Mukai C, Okuno M. Glycolysis plays a major role for adenosine triphosphate supplementation in mouse sperm flagellar movement. *Biology of Reproduction*. 2004;71(2):540–547.

38. Okuno M, Brokaw C. Inhibition of movement of tritondemembranated sea-urchin sperm flagella by Mg2+, ATP4−, ADP and P1. *Journal of Cell Science*. 1979;38:105–123.

39. Assouline-Cohen M, Ben-Porat H, Beitner R. Activation of membrane skeleton-bound phosphofructokinase in erythrocytes induced by serotonin. *Molecular Genetics and Metabolism*. 1998;63:235–238.

40. Kemp R. Foe L. Allosteric regulatory properties of muscle phosphofructokinase. *Molecular and Cellular Biochemistry*. 1993;57(2):147–154.

41. Coelho W, Costa K, Sola-Penna M. Serotonin stimulates mouse skeletal muscle 6-phosphofructo-1-kinase through tyrosinephosphorylation of the enzyme altering its intracellular localization. *Mol Genet Metab*. 2007;92(4):364–370.

42. Bandivdekar A, Segal S, Koide S. Binding of 5-hydroxytryptamine analogs by isolated Spisula sperm membrane. *Invertebrate Reproduction & Development*. 1992;21(1):43–46.

43. Stephens R, Prior G. Dynein from serotonin-activated cilia and flagella: extraction characteristics and distinct sites for cAMP-dependent protein phosphorylation. *J Cell Sci*. 1992;103(4):999–1012.

44. Safarinejad M. Sperm DNA Damage and Semen Quality Impairment After Treatment With Selective Serotonin Reuptake Inhibitors Detected Using Semen Analysis and Sperm Chromatin Structure Assay. *The Journal of Urology*. 2008;180(5):2124–2128.

45. Hernández G, Chirinos M. Proteins from male and female reproductive tracts involved in sperm function regulation. *Zygote*. 2019;27(1):5–16.

46. Tabibzadeh S, Sun X. Cytokine expression in human endometrium throughout the menstrual cycle. *Hum Reprod*. 1992;7:1214–1221.

47. Saucedo L, Sobarzo C, Brukman NG. Involvement of fibroblast growth factor 2 (FGF2) and its receptors in the regulation of mouse sperm physiology. *Reproduction*. 2008;156:163–172.

48. Hernández-Corredor L, Camargo-Rodríguez O, Silva-Torres A, et al. Effects of cryopreservation on sperm subpopulations in goats. *Rev Inv Vet Peru*. 2008;29(3):882–893.

49. Buzón A. *Kinetic analysis of the horse's sperm under the Sperm Class Analyzer system*. Spain: University of Cordoba; 2013. p. 173.

50. Marti J, Aparicio I, García-Herreros M. Head morphometric changes in cryopreserved ram spermatozoa are related to sexual maturity. *Theriogenology*. 2011;75(3):473–481.

51. Tuncer P, Umut T, Halil E, et al. Effects of different doses of trehalose supplementation in egg yolk extender in frozen–thawed Angora buck semen. *Small Ruminant Res*. 2013;4:12.

52. Sepúlveda K, Celis-Alba D, Silva-Torres A, et al. *Effect of a diluent based on coconut water on the morphometry and sperm motility of sheep semen*. USA: National Agricultural And Agro-Industrial Symposium; 2018;1:39–43.

53. Bravo J, Montanero J, Calero R, et al. Relationship between subjective and computerized variables of the moronic sperm movement. *Arch Zootec*. 2011;60(232):1087–1094.

54. Cox F, Alfaro V, Montenegro V, et al. Computer-assisted analysis of sperm motion in goats and its relationship with sperm migration in cervical mucus. *Theriogenology*. 2006;66(4):860–867.

55. Kemp R, Foe L. Allosteric regulatory properties of muscle phosphofructokinase. *Molecular and Cellular Biochemistry*. 1993;57:147–154.

56. Kozdrowski R, Dubiel A, Bielas W. et al. Two Protocols of Cryopreservation of Goat Semen with the Use of Computer-Assisted Semen Analysis System. *Acta Vet Brno*. 2007;76:601–604.

57. Kumar N, Kant A. Trends of male factor infertility, an important cause of infertility: A review of literature. *J Hum Reprod Sci*. 2015;8(4):191–196.

58. Peña A, Linde-Forsberg C. Effects of Equex, one or two step dilution and two freezing thawing rates on post-thaw survival of dog spermatozoa. *Theriogenology*. 2000;54(6):859–875.

59. Quintero-Moreno A. *Study on the dynamics of sperm populations in horse, pig and rabbit semen*. Spain: Autonomous University of Barcelona. Veterinary School; 2003. p. 64.