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 disvalues (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

Introduction

Platelets are derivatives of megakaryocytes, have o 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). The plasma Rich platelets (PRP) is a concentrated fraction of platelets higher than basal levels, researchers such as Anitua et al., and Rodriguez et al. 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 (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

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The 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 Sprose® (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

<table>
<thead>
<tr>
<th>Tube</th>
<th>PRP</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh</td>
<td></td>
<td>PRP</td>
</tr>
<tr>
<td>NaCl</td>
<td></td>
<td>CPPr</td>
</tr>
<tr>
<td>Citrated</td>
<td>Poor</td>
<td>CPp</td>
</tr>
<tr>
<td>NaCl</td>
<td></td>
<td>CPPr</td>
</tr>
<tr>
<td>Rich</td>
<td></td>
<td>CPPr</td>
</tr>
<tr>
<td>EDTA</td>
<td>Poor</td>
<td>CPP</td>
</tr>
<tr>
<td>Rich</td>
<td></td>
<td>CPPr</td>
</tr>
</tbody>
</table>

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 mm², 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. Briefly, spermatozoa with mean 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.

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

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Fresh</th>
<th>NaCl</th>
<th>CPp</th>
<th>CPPr</th>
<th>Epp</th>
<th>EPr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Large</td>
<td>9.4±0.06a</td>
<td>9.40±0.01c</td>
<td>9.74±0.03d</td>
<td>9.75±0.05e</td>
<td>9.83±0.12f</td>
<td>9.94±0.08b</td>
</tr>
<tr>
<td>Width</td>
<td>5.34±0.03c</td>
<td>5.04±0.005f</td>
<td>5.57±0.01g</td>
<td>5.56±0.05j</td>
<td>5.63±0.05k</td>
<td>5.67±0.06l</td>
</tr>
<tr>
<td>Area</td>
<td>43.84±0.46h</td>
<td>39.66±0.07h</td>
<td>45.75±0.63i</td>
<td>46.74±0.31h</td>
<td>46.91±0.79h</td>
<td>48.64±0.36i</td>
</tr>
<tr>
<td>perimeter</td>
<td>26.3±0.14d</td>
<td>25.3±0.04c</td>
<td>26.9±0.04d</td>
<td>27.48±0.13e</td>
<td>27.53±0.27f</td>
<td>28.63±0.18g</td>
</tr>
</tbody>
</table>

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. In contrast, a correlation between certain variables of mobility, abnormalities, and integrity of the acrosome can predict fertilizing capability after cryopreservation. While Evans et al. and Leboeuf et al. 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. However, Muiño-Otero et al. concluded that the fastest and most progressive sperm resist the damaging effect of cryopreservation better (Table 3).
Table 3 Mean and standard error of the descriptors of sperm motility according to treatment performed on the sheep evaluated

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Fresh</th>
<th>NaCl</th>
<th>CPp</th>
<th>CPr</th>
<th>EPp</th>
<th>EPr</th>
</tr>
</thead>
<tbody>
<tr>
<td>VCL</td>
<td>118.04±1.39&lt;sup&gt;a&lt;/sup&gt;</td>
<td>112.92±1.44&lt;sup&gt;a&lt;/sup&gt;</td>
<td>106.50±0.93&lt;sup&gt;a&lt;/sup&gt;</td>
<td>113.56±1.26&lt;sup&gt;a&lt;/sup&gt;</td>
<td>113.62±1.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>125.34±1.18&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>VSL</td>
<td>65.97±1.30&lt;sup&gt;a&lt;/sup&gt;</td>
<td>58.97±1.19&lt;sup&gt;b&lt;/sup&gt;</td>
<td>50.97±0.71&lt;sup&gt;c&lt;/sup&gt;</td>
<td>59.92±1.03&lt;sup&gt;c&lt;/sup&gt;</td>
<td>62.98±0.98&lt;sup&gt;c&lt;/sup&gt;</td>
<td>71.62±1.12&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>VAP</td>
<td>74.10±1.17&lt;sup&gt;a&lt;/sup&gt;</td>
<td>71.12±1.18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>65.57±0.71&lt;sup&gt;a&lt;/sup&gt;</td>
<td>74.16±1.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>74.17±0.95&lt;sup&gt;a&lt;/sup&gt;</td>
<td>83.15±1.04&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>LIN</td>
<td>53.46±0.07&lt;sup&gt;b&lt;/sup&gt;</td>
<td>50.48±0.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>47.21±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>51.52±0.05&lt;sup&gt;c&lt;/sup&gt;</td>
<td>52.99±0.05&lt;sup&gt;c&lt;/sup&gt;</td>
<td>55.76±0.06&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>STR</td>
<td>82.33±0.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>78.25±0.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>74.13±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>76.82±0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>79.79±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>81.34±0.05&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>WOB</td>
<td>61.19±0.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>61.33±0.05&lt;sup&gt;c&lt;/sup&gt;</td>
<td>60.66±0.03&lt;sup&gt;c&lt;/sup&gt;</td>
<td>63.70±0.04&lt;sup&gt;c&lt;/sup&gt;</td>
<td>63.06±0.04&lt;sup&gt;c&lt;/sup&gt;</td>
<td>64.89±0.04&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>ALH</td>
<td>3.95±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.98±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.91±0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.88±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.88±0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.01±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>BCF</td>
<td>7.47±0.10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.58±0.09&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.84±0.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.26±0.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.49±0.07&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.29±0.08&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

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., 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<sup>2+ </sup> and calmodulin. Progressive sperm motility is associated with an increase in intracellular cation in sperm. The research suggests that the use of PRP containing serotonin Metcalf et al., induces the increase in the VCL values, achieving values similar to the study by Jiménez-Trejo et al., 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-Guillén et al., 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 cycle. 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, 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. Moura & Memili determine that the angiotensin converted enzyme (ACE) is a seminal component related to the kallikrein system. ACE catalyzes the formation of angiotensin II and protects sperm receptors, intensifying motility-related events. In the morrung, the activity of the ACE in the seminal plasma has a positive correlation with sperm concentration and fertility. In bovine seminal plasma ACE inhibits the decreasing number of sperm with progressive motility and inhibits the acrosomal reaction after in vitro training.

According to Miki there is growing evidence that ATP supports sperm motility is generated by glycolysis, which takes place throughout the main piece. 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. 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. In addition, the activity of PFK correlates with a complete glycolytic pathway in the muscle. Serotonin increases glucose consumption in skeletal muscle and suggests that this neurohormone can regulate the metabolism of cellular energy, therefore, in sperm, it is likely that serotonin plays a similar role. The study by Jiménez-Trejo 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. 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.

Researchers like Hernández & Chirinos 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. 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. 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. 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 EPr and EPp 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. However, the functionality parameters of the membranes and DNA integrity showed no changes in the normal values.

Authors such as Tuncer et al. 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 ovinos in the tropics, no significant differences were observed as reported by Rubio-Guillen, but if they are below the data presented by Sepúlveda et al., 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.

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 favored by the action of the fibroblastic growth factor that induces sperm motility with respect to fresh semen. This process seems to be effective in the selection of fast sperm, being cheaper and easier than adding plasma Rich of platelets as a biopharmaceutical is highly the internal fluids of the sperm cell in sheep. It can be concluded that different treatments explains the change of the spermatic membranes, of adaptation to the medium, the temperature on the membranes and DNA integrity showed no changes in the normal values.

Acknowledgments

None.

Conflicts of interest

Author declares that there are no conflicts of interest.

References


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