ANIMAL HEALTH

Variation of Seminal Quality Due to Systemic Bacterial Infection in Blackbelly Sheep

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Abstract

Background: Increase in body temperature during feverish states caused by diseases, prolonged droves, or stress, affect seminal quality. The aim of this work was to evaluate the alterations of the spermograms of individuals affected by a systemic bacterial infection, and subsequent recovery after therapy with antibiotics.

Methods: This case was presented in two ovine males (Blackbelly), belonging to the flock of the Center for Research, Postgraduate Education, and Preservation, at the Amazon University. The semen was collected every week using an artificial vagina. In March 2018, the two males showed different pathologies. The treatment was applied on the same day the symptoms were detected, without etiological identification of the pathogenic agents. Then, seriated semen collections were made for two months, once a week, twice a day, at 9:00 and 11:00 h, with a 10-15 min between them. Overall, 16 samples were collected from each ram.

Results: The pH of the ram ejaculates varied from 7.3 before the treatment and during recovery to 6.1 at the most critical moment of infection. The volume of the ejaculates also varied between 1.15 ± 0.19, and 1.00 ± 0.15 ml, respectively ($P \leq 0.001$). The concentration of the ejaculate was 3 000 (500-6 000) x106/ml and 2 200 (800-6000) x 106/ml, respectively. Both rams showed the same behavior in terms of color, and individual and progressive mass motility, affected since the second collection on, to the last one, until they slowly began to recover their normal values.

Conclusions: The greatest impact on seminal quality was observed during the first 7-8 samples from the two clinical cases. Therefore, all the parameters analyzed were affected, coinciding with the duration of the spermatogenesis cycle.

Key words: *Wool sheep, sperm quality, systemic antibiotic, Amazon*

Introduction

In the Amazon, artificial insemination (AI) in ovines ensures increases in the genetic gain rate of the progeny, by male and year. It helps with transportation of the genetic material without droving animals that can be exposed to sanitary risks, and it improves service and data recording (Castelo *et al*., 2008; Ribeiro-Peresa *et al*., 2014; Villa-Duque *et al.*, 2016). Some of the disadvantages are service errors, spread of disease due to inadequate controls, higher costs, and the need to hire skilled personnel (Bernardi *et al*., 2011).

Despite all the benefits, the implementation of AI in ovines is not so commonly practiced as in other domesticated species. The fertility percentages using fresh semen are acceptable (over 60%), though irregular, but the utilization of cryopreserved semen stays below 40% (Perry *et al.*, 2010). The success of AI involves several aspects, it is associated to the ewes (management, seasonality, and genital morphology), and the rams (seasonality, semen quality and/or preservation) (Salamón and Maxwell, 2000; Santiani *et* *al.*, 2007; Barbas *et al.*, 2013), husbandry (environmental conditions and sanitary state, handling), as well as technical factors (insemination site, quantity of spermatozoa/doses, and staff skills) (Anel *et al.*, 2006).

In ovines, the light hours (negative photoperiod) and temperature are the most influential environmental factors on seminal quality. Lower ejaculate quality may last between 15 days and 2 months, following exposure to high temperatures (Carmenate *et al.*, 1982; Lincoln, 1992; Manco *et al.*, 2000). Lozano-Marquez *et al.* (2016) demonstrated that sheep from different breeds are sensitive to the environmental conditions of the High Colombian Tropics. Besides, these environmental stress agents increase scrotal temperature, thus affecting thermal regulation, with a negative impact on variables volume, concentration, individual and progressive motility, viability, and animal morphology. Likewise, during inflammatory processes, testicles undergo a rise in temperature of at least 3 °C, and a decline of 2-3 °C in scrotal temperature gradient (Cisale *et al.*, 1999). Other factors that may influence on temperature increases are fever caused by diseases, prolonged droves, or stress (Simonetti *et al.*, 2014).

The aim of this work was to evaluate the alterations of fertility in face of systemic bacterial infection, and their subsequent recovery after therapy with antibiotics.

MATERIALS AND METHODS

This case involved two hair ovine males (Blackbelly), in 2018. One was named Justin (40 months old), with a body condition of 2.3, weighing 48 kg; the other was named Sambo (44 months old), with a body condition of 2.2, weighing 55 kg. The animals belonged to the flock of the Center for Research, Postgraduate Education, and Preservation, at the Amazon University. The facility is located in Canton Arosemena Tola, province of Napo (Ecuador), km 44, via Puyo-Tena (coordinates: S 01° 14.325´; W077° 53.134´), with 3 ha of grassland used for ovine milk production. The climate is tropical, with precipitation values of 4 000 mm/year, an average relative humidity of 80%, and varying temperature values between 15 and 25 °C. The topography comprises slightly wavy terrains without steep slopes, spread along large natural plateaus. Altitude varies between 580 and 990 m, above sea level. Although the soils are very heterogeneous, most are originated from river sediments dragged from the Andean regions of the country. CIPCA owns five sheep in 0.5 ha, which are fed on *Brachiaria decumbens* (Dallis grass) and *Brachiaria brizantha* (Marandú grass); the animals are stabled at nights with a mineral-supplemented diet. The semen is collected weekly, using the artificial vagina technique, which is a fast method that prevents stress in the rams, and guarantees seriated collections (Aisen, 2004). In March 2018, the two males were observed to show different pathologies.

Justin: During the routine examination prior semen collection (Robles, Updates on Ovine Production, 2015, INTA) the ram showed an unusual behavior, so a detailed semiological examination was performed right away. Clinical characteristics: Heart beat (HB) was 90/min, respiratory frequency (RF) was 18/min, and temperature was 40 ºC. A purulent secretion was observed on the skin and tissues around the scrotum during the general physical examination. According to the symptoms and fever signs combined with inflammation of the ganglia, the assumed diagnostic was septicemic infection due to bacterial contamination following myiasis by fly larvae (*Cochliomyia hominivorax*), located on the scrotum. The treatment included antibiotics and non-steroid anti-inflammatories: a combination of G Benzathine Penicillin, G Procain Penicillin, G Potassium Penicillin, Streptomycin, and Sodium Diclofenac Class© (Laboratory CCLabs). Recommended dose of the product: 1 mL/15 kg of weight. Dose administered: 3.20 mL deep intramuscular injection on three straight days. Besides, treatment of the affected area was made with spraying and baths of iodofore solution and repellents, daily, until total recovery.

Sambo: A thorough semiological examination was performed after detection of claudication. Clinical characteristics: HB: 88/min, RF: 19/min, Temperature 39.8ºC. Limping was evident, with symmetrical bilateral and hyperemic swelling of the heel bulbs, extending to the adjacent toes. A smelly caseous tissue was observed to have caused slight harm on the hoof. The assumed diagnostic was local infection of the right hind limb due to claudication combined with inter-digital infection in the foot, based on the symptoms and signs of fever, inflammation of the crown, gasping, limping, and anorexia. The treatment was based on antibiotics and non-steroid anti-inflammatories: a combination of G Benzathine Penicillin, G Procain Penicillin, G Potassium Penicillin, Streptomycin, and Sodium Diclofenac Class© (Laboratorio

CCLabs). Recommended dose of the product: 1 mL/15 kg of weight. Dose administered: 3.6 mL deep intramuscular injection for five straight days. Topical cleansing of the affected limb with iodophors and repellents, daily, until recovery.

The treatment was applied on the same day the symptoms were detected, without etiological identification of the pathogenic agents. Then, seriated semen collections were made for two months (03/31/2018 to 05/31/2018), once a week, twice a day, at 9:00 and 11:00 h, with a 10-15 min interval between them. Overall, 17 samples were collected from each ram.

The following seminal variables were analyzed: Volume (VOL) mL, pH, color (Evans and Maxwell, 1990), concentration (number of spermatozoa x10^8/mL), mass motility (scale: 0-5), individual motility (scale: 0-5), progressive motility (scale: 0-5), Mortality (%) and Morphology.

All the evaluations were performed by the same person to prevent reading errors. The artificial vagina protocol for semen collection, and general animal handling are part of a PhD thesis approved by the Bioethics Commission at the Faculty of Veterinary Science, National University of Rosario, Resol. CD N° 126/16.

The averages and standard errors were obtained for variables lumen and concentrations in all the collections. The existence of significant differences was checked through the application of simple analysis of variance. JMP, version 5.0, for Windows (JMP®, SAS Institute, 2003), was used for statistical analysis. Descriptive analyses were performed to variables motility, lethality, and morphology.

RESULTS AND DISCUSSION

The pH of the ejaculates from both rams showed the same behavior along the pathology, which varied from 7.3 before the treatment and during recovery, to 6.1 at the most critical moment of infection. The color of semen from the first collection was milky-white, then it varied, from the second through the thirteenth collections (gray and brown indicating infection), and turned normal from the fourteenth sample collection on. Sambo produced a higher average volume of semen than Justin (1.15 ± 0.19, and 1.00 ± 0.15 ml, respectively), with significant differences ($P \leq 0.05$) (Fig. 1). Comparison of each ejaculation volume also showed significant differences, except for three samples (9, 11, and 13), which were similar. The ejaculate volume from each individual during the treatment showed a significant decline, then it recovered from the thirteenth sample on.

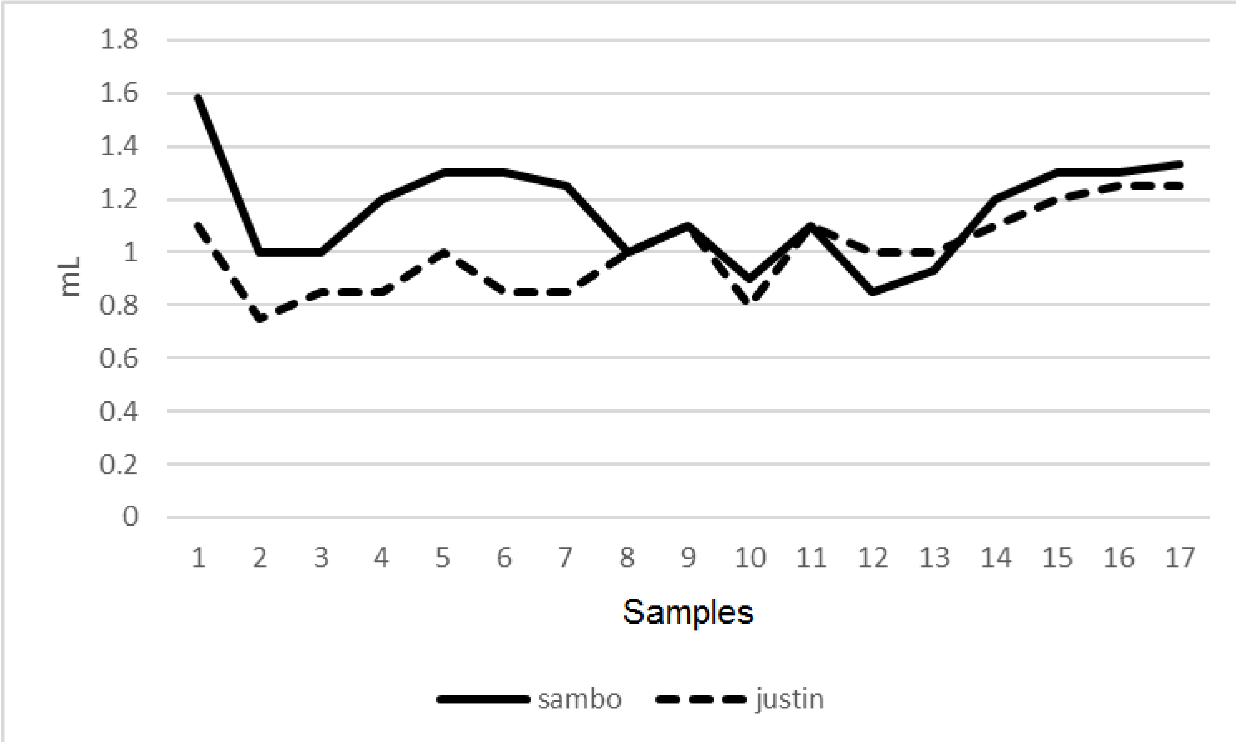

Fig. 1. Average volume in different samples of the ejaculate (mL) of the two hair rams at the two sample collection times

The concentration of Sambo's ejaculates was 2729±494 x106/ mL, with a slight variation in relation to Justin's 2819±494 x106/ mL. No significant differences were observed (P ≥ 0.05) (Fig. 2).

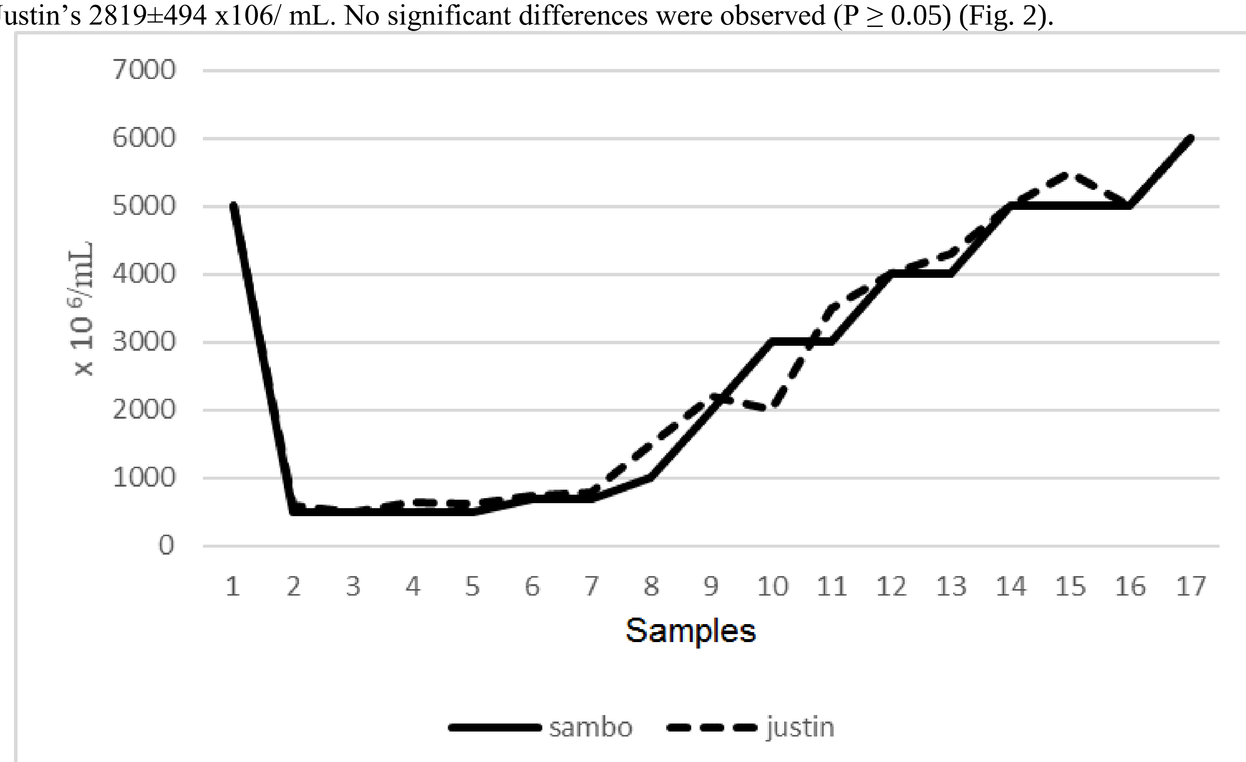

Fig. 2. Concentration x 10^6/mL in different samples of the two hair rams in the two sample collection times

In figures 3 and 4, the ejaculates from both rams showed the same behavior in terms of mass, individual, and progressive motility. They were affected from the second collection on to the tenth, then they slowly began to recover their normal values.

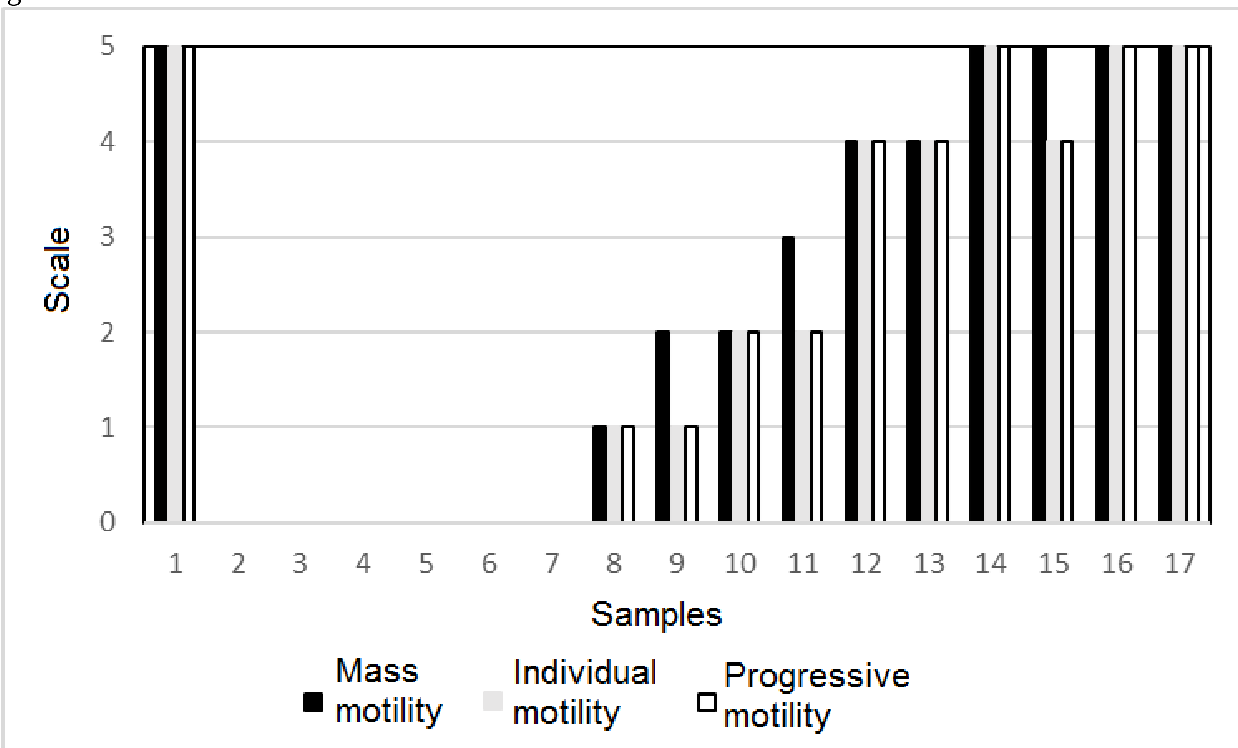

Fig. 3. Justin's motility values, mass (0-5), individual (0-5), progressive (0-5) during collections

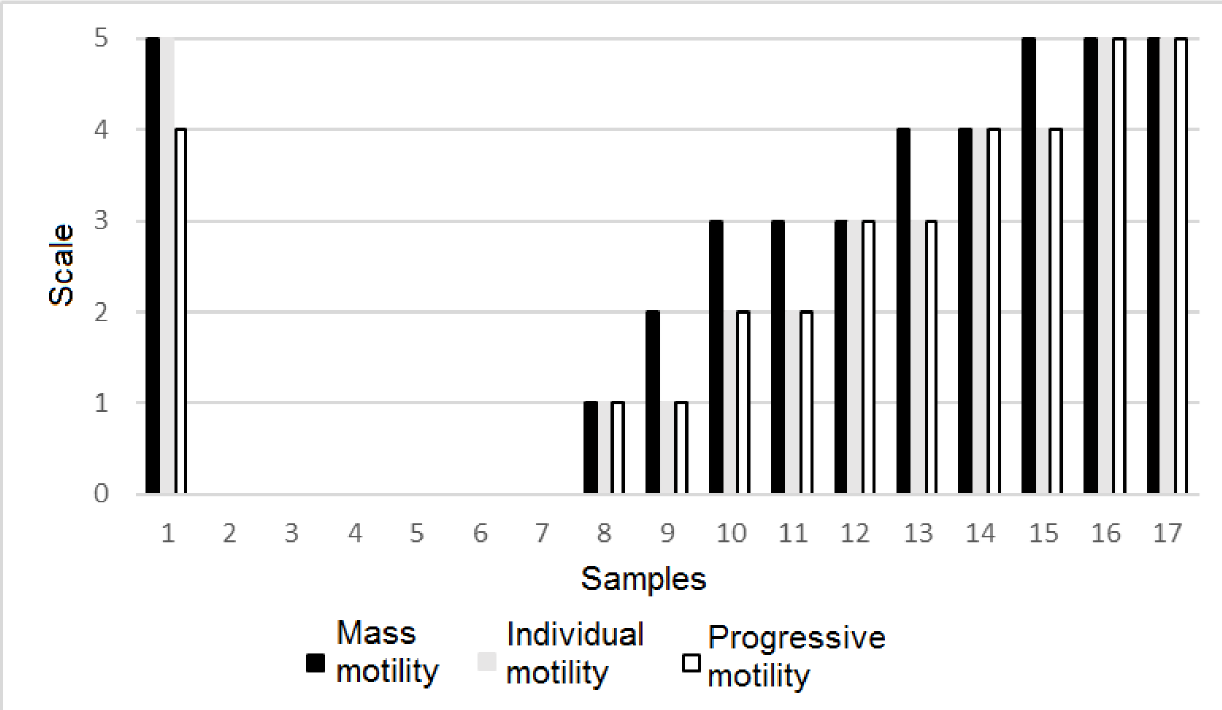

Fig. 4. Sambo's motility values, mass (0-5), individual (0-5), progressive (0-5) during the collections.

Both rams underwent the same behavior in relation to sperm viability percentage, the graphs overlap. At the beginning of the treatment, Sambo showed 2%, and Justin 5% viability. The two rams increased mortality to 100%, until the last collection, then they slowly recovered the normal values from the eighth collection on, reaching the initial values at the last collection (Fig. 5). Likewise, the percentage of morphological alterations at the tails of spermatozoa underwent an identical behavior.

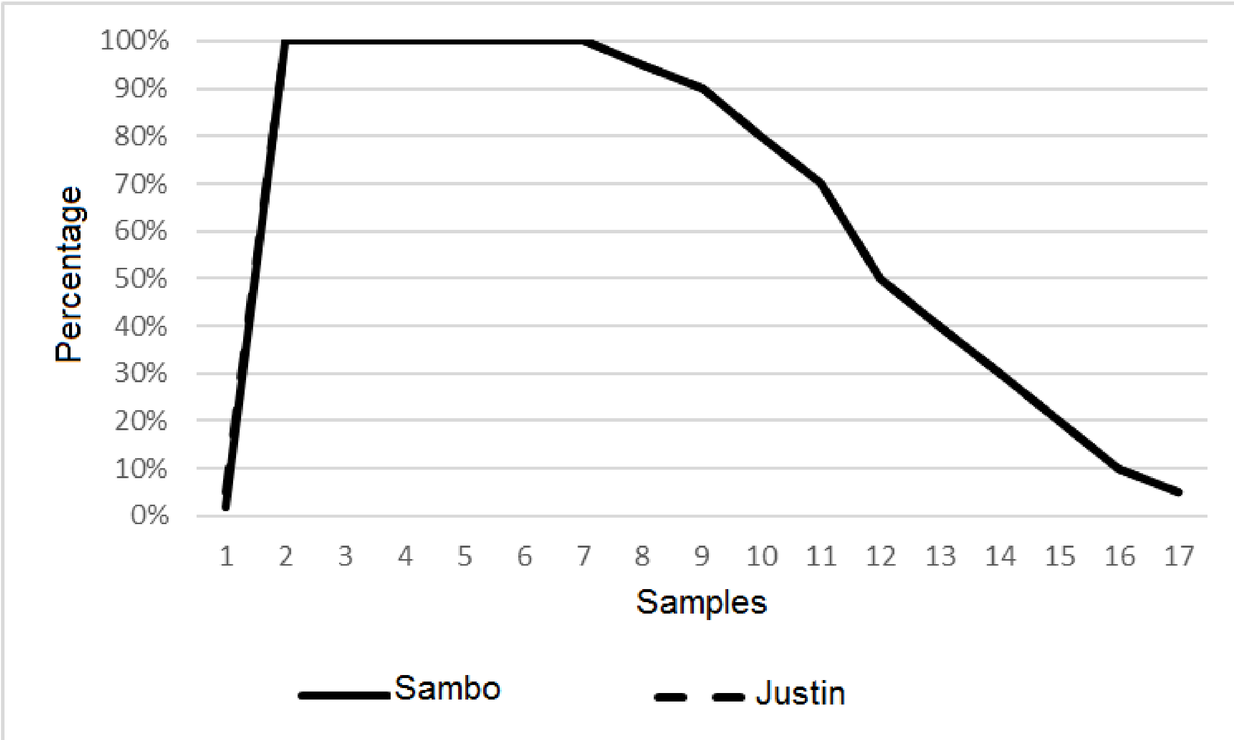

Fig. 5. Mortality percentage of Sambo and Justin during the collections

The ejaculate volume was similar to the one described by Maza Gamboa *et al.* (2015) in Pelibuey individuals, with similar and slightly lower body condition and weight, compared to the reports made by Carrillo-González and Hernández (2015) in native Colombian ovines. Similarly, the sperm concentrations found in the first sample and the last ejaculate samples coincided with the reports published by Maza Gamboa *et al.* (2015), but were higher than the descriptions made in native born sheep (Carrillo-González and Hernández, 2015).

Alves *et al.* (2016), and Escobar *et al.* (2019) said that regardless of the cause of hyperthermia, the increase of testicle temperature reduces seminal quality, particularly in terms of sperm concentration and motility. The authors reported that the recovery of normal values post hyperthermia occurs between 35 and 62 days, coinciding with the duration of spermatogenesis in the species evaluated. In this paper, the pH, viability, and the average values of the ejaculates were affected. Normalization was observed around day 50, which coincides with spermatogenesis in sheep, around day 49 (Hafez and Hafez, 2013).

The findings of this study also coincide with the descriptions made by Cisale *et al.* (1999), who observed a recovery of ejaculate quality after 8-18 months of treatment in two sheep affected by scrotal myiasis with a chronical evolution. In this case, the damage of the testicle parenchyma was highly marked, with fibrous proliferation of the interstitial tissue.

Likewise, the deterioration of the seminal parameters evaluated might not only occur thanks to inflammatory and fever processes, but also as a result of the implementation of a systemic antibiotic therapy. In that sense, the results coincide with the descriptions made by Sinha *et al.* (2012), who observed a normalization of seminal quality, approximately 47 days following systemic administration of antimicrobial agents, coinciding with spermatogenesis.

Importantly, the other three Blackbelly sheep at CIPCA underwent the same collection protocol, but none showed changes in seminal quality (unpublished data). This fact helped discard the influence of environmental stressors like high temperature, relative humidity, and normal rainfall in the tropics, on the changes observed in the samples collected from Sambo and Justin (Lozano-Marquez, 2016, Bossois Moura *et al.*, 2019).

Conclusions

The greatest impact on seminal quality was observed during the first 7-8 samples of the two clinical cases. Therefore, all the parameters analyzed were affected, coinciding with the duration of the spermatogenesis cycle. Although the etiological agent was not identified, the pharmacological treatment applied was adequate, since it favored healing in the two cases. Not only did the systemic signs and symptoms disappeared, but also sperm quality was observed to recover.

References

Aisen, E. (2004). *Reproducción ovina y caprina*. Buenos Aires, Argentina: Editorial Inter Médica.

Alves, M. B. R.; De Andrade, A. F. C.; De Arruda, R. P.; Batissaco, L.; Florez-Rodriguez, S. A.; De Oliveira, B. M. M.; Vellone, V. S. (2016). Recovery of normal testicular temperature after scrotal heat stress in rams assessed by infrared thermography and its effects on seminal characteristics and testosterone blood serum concentration. *Theriogenology*, *86*(3), 795-805.

Anel, L.; Álvarez, M.; Martínez-Pastor, F.; García-Macías, V.; Anel, E.; De Paz, P. (2006). Improvement Strategies in Ovine Artificial Insemination. *Reprod. Dom. Anim.*, *41*(2), 30-42.

Barbas, J.; Marques, C.; Baptista, M.; Mascarenhas, R.; Pereira, R.; Cavaco-Gonçalves, S. (2013). Fertilidade de carneiros de raça Saloia com sémen refrigerado ou congelado. *Arch. Zootec*., *62*(1), 303-306.

Bernardi, S. F.; Allende, R.; Mazzeo, R.; Monti, J. y Marini, P.R. (2011). Evaluación de los cambios ocasionados en espermatozoides bovinos por variaciones en el manejo de las dosis durante su manipulación en inseminación artificial. *In. Vet.*, *13*(2), 25-38.

Bossois Moura, A. B.; Nunes De Souza, G.; Zandonadi Brand~Ao, F.; Novita Esteves, S.; Andrade Pantoja, M. H.; Ferreira Da Fonseca, J. *et al.* (2019). Differences in the thermal sensitivity and seminal quality of distinct ovine genotypes raised in tropical conditions. *Theriogenology* *123*(1), 123-131. https://doi.org/10.1016/j.theriogenology.2018.09.037

Carrillo-González, D. y Hernández, H. D. (2016). Caracterización seminal de individuos ovinos criollos colombianos de pelo en el departamento de Sucre. *Rev Colombiana Cienc. Anim*., *8*(2), 197-203.

Carmenate, C., Gamicik, P., & Hernández, J. J. (1982). *Influencia de algunos factores climáticos sobre las características físicas y morfológicas del semen bovino*. La Habana, Cuba: Centro de Información y Documentación Agropecuario.

Castelo, T. S.; Frota, T. R. y Silva, A. R. (2008). Considerações sobre a criopreservação do sêmen de caprinos. *Acta Vet. Bras.*, *2*(1), 67-75. DOI.org/10.21708/avb.2008.2.3.885

Cisale, H.; Rivolta, M. y Fernandez, H. (1999). Semen characteristics of ram after scrotal myiasis. *Vet. Rec.*, *145*(1), 642-643. DOI: 10.1136/vr.145.22.642

Escobar, E.; Lopes, S.; Malavolta, C.; Bernera Ramalho, J.; Missio, D.; Fernandes Pinto, H. et al. (2019). Effect of g-oryzanol on testicular degeneration induced by scrotal insulation in rams. *Theriogenology, 128*(1), 167-175. DOI.org/10.1016/j.theriogenology.2019.01.041

Evans, G.; Maxwell, W. M. C. (1990). *Inseminación Artificial de ovejas y cabras*. Zaragoza, España: Editorial Acribia S.A.

Hafez, E. S. E. y Hafez, B. (Eds.). (2013). *Reproduction in farm animals*. Philadelphia, EE.UU.: John Wiley & Sons.

Lincoln, G. A. (1992). Photoperiod-pinealhypothalamic relay in sheep. *Anim. Reprod. Sci.*, *28*(1), 203-217. DOI.org/10.1016/0378-4320(92)90107-O

Lozano-Marquez, H., Caravajal-Serna, M., Manrique-Perdomo, C., Grajales-Lombana, H. A. (2016). Parámetros de calidad seminal y su relación con las variables medioambientales en ovinos bajo condiciones de trópico alto colombiano. *Actas Iberoamericanas en Conservación Animal*, *8*(1), 56-62.

Manco, Y. M.; Leyva, V. V.; Camacho, J. S. y Cueva, S. M. (2014). Efecto de la temperatura escrotal sobre el comportamiento sexual y la calidad de semen de ovinos pelibuey y merino precoz alemán. *Revista de Investigaciones Veterinarias del Perú*, *11*(2), 51-61. https://doi.org/10.15381/rivep.v11i2.7061

Maza Gamboa, J.; Navarrete Sierra, L. F.; Aguiar Loría, A.; Zamora Bustillos, R. y Magaña Sevilla, H. (2015). Calidad seminal en ovinos Pelibuey con inclusión de Hibiscus rosa-sinensis en la dieta. *Nova scientia*, *7*(15), 33-48.

Perry, K.; Haresign, W.; Wathes, D.C. y Khalida, M. (2010). Intracervical application of hyaluronan improves cervical relaxation in the ewe. *Theriogenology, 74*(1), 1685-1690. DOI:10.1016/j.theriogenology.2010.07.008

Ribeiro-Peresa, A.; Munita-Barbosab, L.; Yumi-Kanazawab, M., Mello-Martinsc, M. I.; Ferreira De Souzad, F. (2014). Criopreservación de espermatozoides bovinos extraídos de la cola del epidídimo utilizando los métodos convencional y automatizado. *Arch. Med. Vet.*, *46*(1), 31-38.

Salamon, S. y Maxwell, W. (2000). Storage of ram semen. Anim. *Reprod. Sci.*, *62*(1), 77-111. DOI:10.1016/S0378-4320(00)00155-X

Sinha, C.; Yadav, S.; Yadav, B. y Singh, K. (2012). Effects of enrofloxacin administration on semen quality of barbari bucks. *Journal of Advanced Veterinary Research*, *2*(1), 179-183.

Santiani, A.; Ruiz, L.; Sandoval, R.; Evangelista, S.; Urviola, M.; Catacora, N. *et al.* (2007). Incremento de la tasa de no retorno de celo en ovejas utilizando un antioxidante análogo de super óxidodismutasa (Tempo) durante la criopreservación de semen. *Arch. Latinoam. Prod. Anim.*, *15*(1), 347-351.

Simonetti, L.; Lynch, G.; Mc Cormick, M. (2014). Aspectos reproductivos de los carneros. *Revista de divulgación técnica agropecuaria y ambiental UNLZZ*, *1*(1), 15-20.

Robles, C. (2015, septiembre). *Actualización en Producción Ovina*. Memorias del IX Curso de Producción Ovina, 14 al 18. San Carlos de Bariloche.

Villa-Duque, N.; Amaya-Torres, C. M.; García-Rojas, D.; Nieto-Omeara, N.; Terán-Acuña, N. (2016). Efecto de la manipulación del semen criopreservado de bovinos *Bos Taurus* sobre la integridad espermática. *Rev. Cien. Agri.*, *13*(1), 9-18.

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AUTHOR CONTRIBUTION

Author participation included the following: Conception and design of research: JJL, JJM, data analysis and interpretation: PRM, MLF, redaction of the manuscript: PRM, MLF

CONFLICTS OF INTEREST

None