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Influence of two electroejaculation voltages on the seminal quality of alpacas (*Vicugna pacos*)

Influencia de dos voltajes de electroeyaculación en la calidad seminal de alpacas (*Vicugna pacos*)

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Article Data

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Abstract

The objective of the research was to evaluate the influence of two voltages in the electroejaculation protocols on the seminal quality of alpacas (*Vicugna pacos*). t was developed in the Reproductive Biotechnology Laboratory, Salache Academic Experimental Center, Technical University of Cotopaxi. Eight male alpacas were used and from each one two ejaculates were collected weekly, one using the electroejaculation protocol that used several 10 volt stimuli, and the other using a protocol where the stimuli were 12 volts. The volume of the ejaculated determined itself for direct observation in measuring glass, la concentration; sperm motility, viability and morphology by Neubauer chamber, DM4B YDM6B mass microscope at 10X, Eosin-Nigrosin staining and observation of semen-eosin staining, respectively. Volume, concentration of ejaculates, motility, seminal pathologies were compared according to the electroejaculation protocol by means of the Student's t test for independent samples. The volume of the ejaculates was 0.75±0.20 and 1.10±0.39 mL, the concentration 2.12±1.93 and 2.25±1.89 (x10^6), the sperm mass motility 81. 25±11.81 % and 80.00±14.14 %, mortality 17.50±9.57 and 15.00±10.80 % and morphoanomalies 12.50±6.45 and 13.50±8.01 % in the animals that received electroejaculation with 10 and 12 volts, respectively. There were no significant differences ($p > 0.05$) in the seminal parameters evaluated according to the voltage used in the ejaculation protocol. It is concluded that the increase in voltage in the electroejaculation protocol had no influence on the seminal quality parameters of alpacas

Resumen

La investigación tuvo como objetivo evaluar la influencia de dos voltajes en los protocolos de elecroeyaculación en la calidad seminal de alpacas (*Vicugna pacos*). Se desarrolló en el laboratorio de Biotecnología de la Reproducción, Centro Experimental Académico Salache, Universidad Técnica de Cotopaxi. Se utilizaron 8 alpacas machos y de cada uno se colectaron dos eyaculados con una frecuencia semanal, uno mediante el protocolo EE que usó varios estímulos de 10 V, y en el otro mediante un protocolo donde los estímulos fueron de 12 V. El volumen del eyaculado se determinó por observación directa en probeta graduada, la concentración; motilidad, viabilidad y morfología espermática mediante cámara de Neubauer, microscopio masal DM4B Y DM6B a 10X, tinción Eosina-Nigrosina y observación de la tinción semen-eosina, respectivamente. Se compararon el volumen, la concentración de los eyaculados, motilidad, patologías seminales según el protocolo de EE mediante la prueba de t-Student para muestras independientes. El volumen de los eyaculados fue 0.75±0.20 y 1.10±0.39 mL, la concentración 2.12±1.93 y 2.25±1. 89 (x10^6), la motilidad masal los espermatozoides 81.25±11.81 % y 80.00±14.14 %, la mortalidad 17.50±9.57 y 15.00±10.80 %, las morfoanomalías 12.50±6.45 y 13.50±8.01 % en los animales que recibieron la electroeyaculación con 10 y 12 V, respectivamente. No existieron diferencias significativas ($p > 0.05$) en los parámetros seminales evaluados según el voltaje utilizado en el protocolo de eyaculación. Se concluye que el incremento del voltaje en el protocolo de electroeyaculación no tuvo influencia en los parámetros de calidad seminal de alpacas.

Introduction

Alpacas are domestic animals that belong to the New World group, the Camelidae family, they live mainly in South America[1,2], their breeding constitutes a source of income for the residents of Andean communities in Peru, Bolivia, Chile, Argentina and Ecuador[3].

To accelerate its genetic improvement, it is necessary to use reproductive biotechnologies, which include, among others, collection, evaluation and conservation of semen[4].

There are differences in seminal composition, and a lower concentration of spermatozoa in ejaculates collected by electroejaculation (EE) compared to the other method that involves artificial vagina[5], and including sperm quality evaluation after defreezing which is lower in semen collected by EE[6]. However, it is a viable method to collect ejaculates in males that are not trained or wild species[7].

EE is based on the application of electrical stimuli to the pelvic nerves through a probe inserted into the rectum, thus promoting protrusion of the penis, erection and finally ejaculation[8].

EE for alpaca semen collection was used with very variable results, the ejaculates had little volume (0.1 to 0.5 mL) and high concentration[9]. Later in this species it was widely utilized EE[10-12], it is also used in vicuna[13], llama[14-16].

The voltage used in the EE influences the sperm quality, in alpacas most of the protocols used use 2 to 10 V, which avoids stress in the animals, to obtain good quality ejaculates, according to their volume, concentration and motility, provided that the males are handled correctly prior to the application[10,12,17].

In other species, different voltages are used in the EE, which influence the time to induce ejaculation and volume. In llamas, 2 to 10 V are used and the ejaculate is obtained after 6 min[14,18], 3 to 10 V1[9] were also used with similar results.

In this species, in Bolivia, the ejaculation occurred with a voltage of 18 V, obtaining 0.9 mL of ejaculate[20], in Peru, 2 to 20 V were applied, penile erection occurred at 15 V, while ejaculation required 20 V, 1 mL of ejaculate was collected[21]. In the vicuna, between 2 and 12 V have been used, ejaculation occurred at 6 V[13].

Information on the effect of the voltage used in EE on the seminal quality of alpacas is scarce, due to the lack of a reliable and reproducible methodology for semen collection. The purpose of this work was to evaluate the influence of two voltages in the EE protocol on the seminal quality of alpacas (*Vicugna pacos*).

Materials and methods

The research was carried out between May and August 2020, in the province of Cotopaxi, Lata cunga canton, located between 0° 59' 10" south latitude (LS) and 78° 37' 13" west longitude (LW), at 2739 meters above sea level, with an average temperature of 12 °C, relative humidity of 75 %, average annual rainfall between 500 and 1500 mm, clay soil[22].

The facilities of the Salache Academic Experimental Center (CEASA), Reproductive Biotechnology Laboratory of the Veterinary Medicine, Technical University of Coto Paxi were used.

Eight male alpacas (*V. pacos*) were selected, aged between 3 and 5 years, with 64±2.2 kg of weight, without reproductive problems, of congenital defects, clinically healthy, with feeding based on natural pastures and water ad libitum. Two ejaculates were col-

lected from each of these animals with a weekly frequency with the same number of EE protocols for this purpose; they had the same characteristics.

The animals were supplied to the alpaca wool and genetic improvement project of the Technical University of Cotopaxi by the indigenous communities of the provinces of Cañar, Chimborazo, Cotopaxi and Imbabura, in Ecuador.

The ES (Figure 1) was performed according to the basic principles of the technique described by Director et al.[18], to which the following adjustments were made. i) several 10 V stimuli were used, with electrical stimulation periods of 4 s, 1 s with rest intervals of 1 s, for 4-8 min ii) the stimuli were 12 V, with equal intervals and duration. An ElectroJac 5® electro-ejaculator (Ideal Instruments®, Lansing, USA) was used for this procedure.

Figure 1 Application of EE to alpacas in this research

The ejaculates were collected in 50 mL falcon tubes (Figure 2), protected by an external cover that maintained a temperature of 37 °C, and were transported to the CEASA Reproductive Biotechnology Laboratory in less than 1 h from collection. Samples contaminated with urine were not processed.

Ejaculate volume was determined by direct observation using a graduated cylinder. Seminal concentration, using the Neubauer chamber and motility using a DM4B and DM6B mass microscope (Leica Microsystems AG, USA), at 10X. Both parameters were determined according to the procedures described by Allauca et al.[23].

Sperm viability was determined by staining Eosin-Nigrosin, dead spermatozoa do not they take colorant and appear pink, however, the live ones appear translucent[24].

Sperm morphology was evaluated by observing semen-eosin staining in a scanning electron microscope, the number of morphologically normal and abnormal sperm from according to the recommendations of Pérez et al.[25].

Statistical processing. Descriptive statistics were obtained for each variable. The volume, concentration of the ejaculates, motility and semen pathologies were compared according to the electroejaculation protocol using the t-Student test for independent samples. In all the processes, the statistical package Statgraphics Centurion[26] was used.

Figure 2 Collection of the ejaculate

Results

Table 1 shows the seminal variables evaluated of the EE with 10 and 12 V.

There were no significant differences ($p > 0.05$) in the seminal parameters evaluated according to the voltage used in the ejaculation protocol. However, the volume was greater numerically when the voltage was 12 V. In this group there was a tendency to decrease mortality and morphoanomalies (Table 2).

Table 1 Descriptive statistics of the seminal variables of the alpacas studied

Statisticians	Volume (mL)		Concentration (x10^6)		Mass motility (%)		Mortality (%)		Morphoanomalies (%)	
	10 V	12 V	10 V	12 V	10 V	12 V	12 V	12 V	12 V	12 V
□	0.75	1.10	2.12	2.25	81.25	80.00	17.50	15.00	12.50	13.75
SD	0.20	0.39	1.93	1.89	11.81	14.14	9.574	10.80	6.45	8.01
CV	27.75	35.59	90.87	84.13	14.54	17.67	54.71	72.00	51.63	58.29
Minimum	0.50	0.60	1.00	1.00	65.0	60.00	10.00	5.00	5.00	6.00
Maximum	1.00	1.50	5.00	5.00	90.00	90.00	30.00	30.00	20.00	25.00
Range	0.50	0.90	4.00	4.00	25.00	30.00	20.00	25.00	15.00	19.00

SD: Standard deviation. CV: Coefficient of variation

Table 2 Seminal parameters ($\bar{x}$) of alpacas with two voltages used in the electroejaculation protocol

Parameters	Groups		± EE	p-value
	10 V	12 V		
Volume (mL)	0.75	1.10	0.15	0.16
Concentration (x10^6)	2.12	2.25	0.95	0.92
Mass motility (%)	81.25	80.00	6.51	0.89
Mortality (%)	17.50	15.00	5.10	0.74
Morphoanomalies (%)	12.50	13.75	3.63	0.81

Discussion

The volume of ejaculates, sperm concentration, sperm mass motility, mortality and morphoanomalies are lower than those obtained in alpacas from Peru[27].

The volume was lower than that indicated by Trujillo Bravo[28], who obtained 1.48 to 1.91 in males of the Huacaya Breed from 3 to 5 years of age. This work confirms that alpacas have low volume in their ejaculates[29].

Although alpacas have low sperm concentration[29], the one obtained in this research indicates that the ejaculates have quality to carry out artificial insemination and from them more seminal doses can be obtained, a parameter that will depend on the dilution rate to be used[21].

Individual motility in alpaca is not an indicator of the quality of semen, fresh or cryopreserved[27], however, according to the criteria of Allauca et al.[23], in this investigation the mass and individual motility describe

the ejaculates as good and confirm that South American camelids have little or no individual mobility[29].

Morphoanomalies are related to the size of the spermatozoa, affect fertilization capacity, and can be found in any segment of the spermatozoa[30,31]. The percentage of spermatozoa with abnormal shapes did not differ according to the voltage used in the EE, which is motivated by the fact that these cells present great polymorphism and vary morphologically inter individually in each male of the South American camelids[32,33].

Mortality was lower than that obtained in a study, the collection of semen in alpacas, was done by vaginal aspiration and artificial vagina, the percentage of dead zoosperms was 24.7 and 29.2 %, respectively[34]

Any EE protocol must maintain the viability of the largest number of possible spermatozoa, since this is positively related to acrosomal integrity in vivo and in vitro[35].

The causes of the differences in the seminal parameters determined in this work with the consultants may be motivated by the differences in the edaphoclimatic, physiological and production conditions[8].

The volume, concentration, motility, sperm morphoanomalies vary according to the physiological state of the male, age, race, feeding, frequency collection and psychosexual separation[36].

It is concluded that the increase in the voltage level from 10 to 12 V in the EE protocol had no influence on the ejaculate and seminal quality parameters of alpacas (*V. pacos*)

Source of financing

We must emphasize that the financing of the research Influence of two voltages in the protocols of electroejaculation in the seminal quality of alpacas (*V. pacos*), was carried out with the researchers' own resources.

Conflicts of interest

The authors declare that there were no potential conflicts of interest with respect to the authorship and/or publication of this article.

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Ethical considerations

The protocols used in the study adhere to the European standards indicated in https://ec.europa.eu/environment/chemicals//lab_animals/legislation_en.htm for the use of animals in research.

Authors' contribution to the article

Luis Alonso Chicaiza Sánchez, search for information, study design, sampling, writing of the draft. *Rafael Alfonso Garzón Jarrin*, information search, sample taking, writing and drafting of the document. *Eddy Bryan Masabanda Soria*, processing and analysis of samples in the laboratory. *Juan Ramón García Díaz*, writing and drafting of the document, revision, edition and revision of the manuscript.

Research limitations

It was not possible to establish and validate a methodology for obtaining semen of this species by electroejaculation because of the limited quantity of processed samples of them literature cited.

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