COMPARISON OF COMMERCIAL DILUENTS AND TRACE ELEMENTS ON FRESH ALPACA SEMEN (LAMA PACOS)

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RESUMEN

La reproducción de las alpacas es la materia de interés investigativo en los últimos tiempos, puesto que su condición y morfología no causan impacto a los páramos y territorios de hábitat. La recolección de semen de alpacas macho por vagina artificial es la metodología de mejor extracción del material seminal, sin embargo, los métodos de conservación no se definen en su totalidad, por ello, analizar la conservación de semen en las alpacas macho con la inclusión de oligoelementos y diluyentes comerciales fue el interés en este estudio. Se utilizó 7 alpacas macho con edades reproductiva entre 3 y 5 años y peso promedio de 60 ± 1,64 kg de PV, distribuidos en dos grupos homogéneos experimentales al azar (SSO, semen de alpacas macho sin oligoelementos y SCO, semen de alpacas macho con oligoelementos) para analizar las características macroscópicas y microscópicas sobre la administración de oligoelementos de carácter comercial y sometidas a un procedimiento de dilución con dos tipos de diluyentes comerciales registrados (Triladyl® y AndroMed®) y un diluyente generado en laboratorio (Tris Yema + Glicerol). El proceso de conservación de semen analizado frente a la aplicación de oligoelementos no posee diferencia, pese a que exista heterogénea investigación sobre la conservación de semen. Se recomienda realizar más aportes científicos a la inclusión de oligoelementos y diluyentes sobre el semen fresco de alpacas macho.

Palabras claves: diluyentes, semen de alpaca, oligoelementos, simetría testicular

Abstract: *The reproduction of alpacas is a research subject of interest in recent times since their condition and morphology do not cause an impact on the Andean-habitat territories. The collection of semen from male alpacas by artificial vagina is the methodology used for the best extraction of seminal material. However, conservation methods are not fully defined, therefore, analyzing the semen conservation on male alpacas with the inclusion of trace elements and commercial diluents were the subject of interest in the study. Seven male alpacas were taken, with reproductive ages between 3 - 5 yrs. with an average weight of 60 ± 1.64 kg of BW. Distributed in two random homogeneous experimental groups (SSO, male alpacas semen without trace elements, and SCO, male alpacas semen with trace elements) to analyze the macroscopic and microscopic characteristics of the commercial trace elements administration. In addition, were subjected to a dilution procedure with two types of registered commercial diluents (Triladyl® and AndroMed®) and a laboratory-generated diluent (Tris Yema + Glycerol). The semen conservation process was analyzed and compared to the trace elements application and has no difference, even though there is heterogeneous research on semen conservation. The authors recommended making more scientific contributions to the inclusion of trace elements and diluents in the fresh semen of male alpacas.*

***Keywords**: diluents, alpaca semen, trace elements, testicular symmetry*

INTRODUCTION

The usefulness of alpacas in recent years has spread widely throughout the world. The production of fiber or as exotic animals, make this species one of the most researched production animals in recent times, since its condition and morphology do not cause an impact on the paramos and habitat territories (Vivanco, 2002 and Eguren, 2016). The production of alpacas has grown remarkably in South American countries such as Ecuador, Peru, Bolivia, Chile, and Argentina. Research in nutrition, genetics, and reproduction are in parallel with other species of livestock production. Ecuador is participating in the implementation of new reproductive technology to support research. In addition, the government, within a sustainable livestock plan, focuses on agricultural activities with friendly production models and with the environment that involves alpacas' production in Chimborazo, Tungurahua, and Cotopaxi (Quispe et al., 2009).

The sexual behavior of South American camelids is characteristic of the species, alpacas particularly since their nervous temperament and coital position are determinants when obtaining semen as well as a difficult practice. While the semen collection has drawbacks such as the duration of copulation, the place of semen deposit, type of ejaculation, appearance of the ejaculate, its extreme viscosity, and the difficulty of its handling. This induces the investigation of optimal techniques for the extraction and conservation of semen (Vivanco, 2002; Vaughan et al., 2003).

Semen collection in alpacas is done through vaginal sheaths, urethral fistula, and artificial vagina; however, conservation methods are not fully defined (Vivanco, 2002). For this reason, the need for the analysis of viable reproducers was raised, detailing the best reproductive methodology that allows carrying out an adequate extraction protocol for the optimization of the males and the conservation of semen for genetic replication. The objective was to analyze and implement a protocol for the conservation of semen in male alpacas with the inclusion of trace elements and commercial diluents.

MATERIALS AND METHODS

All animal procedures were performed in accordance with the Ecuadorian guidelines for the protection of experimental animals. In accordance with the legislation and experimental procedures approved by the Ethics Committee on Animal Experimentation of the Republic of Ecuador. The research was carried out at the "Aña Moyocancha" Experimental Station belonging to the Livestock Sciences Faculty of Escuela Superior Politécnica de Chimborazo. The Experimental Station is located at an altitude of 3700 meters above sea level, in the Alausí Canton in Chimborazo Province.

Selection of males and morphological characteristics of their testicles

The normal health conditions (Table 1), phenotypic conditions, optimal body condition (CC, 3 out of 5 scale) and testis morphology allowed the selection of 7 male alpacas with reproductive ages between 3 and 5 years and an average weight of 60 ± 1.64 kg of live weight (PV, table 2). The males were distributed into two homogeneous groups according to their live weight, in each group one of the two experimental procedures was randomly assigned (SSO, male alpacas' semen without trace elements and SCO, male alpacas semen with trace elements).

Table 1. Hematic biometry of selected male alpacas

Item[a]	Male alpacas							References[b]
	1	2	3	4	5	6	7	
	Erythrogram							
Hemoglobin, g/dl	11,3	11,3	9,7	13	10	9,7	10,8	9.2 - 15.2
Hematocrit, %	31	31	30	32	31	30	34	20 - 32
Erythrocytes *10 $^{6/uL}$	3,85	3,85	3,35	4,35	3,45	3,35	3,70	7.1 - 13

MCV, fL	90,9	90,9	89,5	91,9	89,8	89,5	90,4	18 - 34
MCH, pg	15,3	14,3	15,9	10,8	10,9	14,9	13,2	8 a 16
MCHC, g/dl	32,2	32,2	32,3	32,3	32,2	32,3	32,3	37 - 57
Leukogram								
Leukocytes*$10^{3/uL}$	6,9	7,5	7,5	9	7,9	8,8	7,9	4,5 - 19
Neutrophils, %	62	56	60	52	53	51	55,7	32 - 71
Lymphocytes, %	35	41	37	45	44	46	41,3	8 a 45
Monocytes, %	1	1	1	1	1	1	1	0 a 7
Eosinophils, %	1	1	1	1	1	1	1	0 a 36
Basophils, %	1	1	1	1	1	1	1	0 a 6

[a] MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration. [B] Specific analysis for camelids by RT-7600 FORVET brand RAYTO

In the measured testicles, a non-pendulous scrotum was considered, without a defined neck and forming a sub-anal protuberance in the animals. In addition, an ovoid shape and oriented dorsoventrally, with the head of the epididymis in the ventral plane and the tail of the epididymis in the dorsal plane. Testicular length and width of male alpacas were measured with a Mitutoyo ® caliper (Mitutoyo Corporation, Aurora, IL, USA) with a minimum resolution of 0.01 mm, and scrotal circumference was measured with a tape measure. For the complete blood count analysis, 1.5 ml of blood was taken into Eppendor tubes containing EDTA. The samples were analyzed in the RAYTO brand RT-7600 FORVET equipment specific for camelids.

The males selected for seminal extraction by an artificial vagina underwent a foreskin cleaning, and the external and internal hair areas were cleaned and trimmed, together. They trained for a week with the help of a mount in the shape of a female alpaca sitting in a mating position. The mannequins were incorporated with the artificial vagina for South American camelids.

Table 2. Testicular symmetry of male alpacas

Male Alpaca	Liveweight	Position	Testicular shape	Length	Width	Scrotal Circumference
1	62	Ventral back	Ovoid	4,13	2,38	22,1
2	58	Ventral back	Ovoid	4,00	2,33	20,1
3	57	Ventral back	Ovoid	4,25	2,35	21,0
4	61	Ventral back	Ovoid	4,38	2,43	20,1
5	60	Ventral back	Ovoid	4,38	2,45	20,3
6	60	Ventral back	Ovoid	4,36	2,41	21,9
7	61	Ventral back	Ovoid	4,20	2,3	22,0

Seminal Extraction

The artificial vagina was prepared with a rubber sleeve inside the vaginal sleeve, its ends were folded to the outside and fixed with pressure garters. A funnel was placed at one end and a Falcon tube was attached to its end, the space between the casing and the tube was filled with hot water at 45 °C. Air was incorporated through the valve to generate the necessary pressure and extend the sleeve towards the center of the tube, decreasing the lumen and simulating the female alpaca's vagina.

The artificial vagina was covered by gel pads at 80 °C, the device consisting of a thermostat (Ceramic Heat Thermostat - INK Bird) and a heat source (Frost King HC30A-Kit of automatic heating cables) was assembled. The male was placed next to the dummy in the seminal extraction room, while the internal temperature of the lubricated artificial vagina was verified to be between 39 and 41 °C. Seminal samples (drip ejaculation) were collected in Falcon tubes, to be immediately transferred to the Laboratory of Animal Reproduction and Artificial Insemination of the Faculty of Livestock Sciences – ESPOCH, for the evaluation of seminal characteristics such as pH, color, volume, motility, mortality, viscosity, abnormalities, vitality and sperm concentration. The seminal material collection was carried out twice a week for each extraction method following the methodology described by Vivanco (2002).

Trace element application

The macroscopic and microscopic characteristics of all the samples were immediately evaluated for the data collection of the SSO experimental group. Subsequently, 5 ml of Thoromangan® was administered as a source of commercial trace elements (Table 3, registered product ®) to each semen sample to evaluate the macroscopic and microscopic characteristics of the SCO experimental group. Fresh semen evaluations were performed at seminal material extraction intervals of 2, 4, and 6 days. It was repeated at intervals of days prior to the application of the trace elements with the same frequencies, in the case of diluted semen it was carried out at intervals of 1h until zero motility. The sperm viability of the diluted semen was evaluated considering individual motility as the only variable, each of the treatments was carried out with all the experimental animals.

Table 3. Chemical composition of the trace elements source (Thoromangan®) for preserving male alpacas' semen.

Trace Elements	Quantity (100 ml solution)
Manganese chlorate (g)	0,340
Potassium iodide (g)	0,785
Cobalt oxychloride (g)	0.255
Sodium iodide (g)	0,785
Cerium chlorate (g)	0,255
Resublimed iodine (g)	0,300
Sodium Chloride (g)	0,215

Fresh semen dilution method

Seminal samples were prepared and submitted to a dilution procedure with three types of diluents for the preservation of fresh semen. The preparation of the diluents used in the experimental procedure were two registered commercial diluents (Triladyl® and AndroMed®) prepared according to the technical recommendations of the commercial houses and a diluent generated in the laboratory (Tris Yolk + Glycerol).

The chemical composition of the diluent Tris Yolk + Glycerol is shown in table 4. The diluent was prepared following the modified dissolution protocols of Cueto et al. (2016). TRIS, $C_6H_{12}O_6$, $C_6H_8O_7$ (0.44 g, 0.06 g and 0.24 g, respectively) and distilled water were placed in a beaker to make 12 ml of solution. The dissolution was carried out by shaking it at an average temperature of 35 °C, immediately adding penicillin, streptomycin sulfate, fresh egg yolk (100,000 IU, 0.66 g and 1.8 ml, respectively).

Table 4. Chemical composition of the diluent Tris Yolk + Glycerol

Item	Content (100 ml)
Tris, (g)	0,44
Glucose, (g)	0,06
Citric Acid, (g)	0,24
Egg yolk, (ml)	1,8
Penicillin, (UI)	100000
Streptomycin Sulfate, (g)	0.66
Glycerol, (ml)	0,6
Distilled water to completion, (ml)	12,0

The solution was transferred to a beaker and stirred magnetically at 37 °C. The solution was filtered through a seminal collection cover until a volume of 6 ml of total dilution was obtained. In addition, glycerol was added dropwise and homogenized on a magnetic stirrer until reaching 37 °C. The final pH of 6.8 was immediately measured and the dilution was kept in a test tube at 37°C in a water bath.

AndroMed® diluent contains Tris, citric acid, sugars, antioxidants, buffers, glycerin, high purity water, and antibiotics (tylosin, gentamicin, spectinomycin, lincomycin). AndroMed® was diluted with distilled water at 35 °C in a 1:4 ratio. 2.4 ml of AndroMed® and 9.6 ml of distilled water were mixed and shaken until the dilution was well homogenized. The dilution was filtered through a seminal collection bag filter, obtaining 6 ml of total dilution. The final pH of 6.80 was immediately measured and the dilution was kept in a test tube at 37°C in a water bath.

The Triladyl® diluent contains TRIS, citric acid, sugar, buffers, glycerin, antibiotics, and extremely pure water. Its chemical composition is shown in Table 5. The diluent preparation is combined in a 1:3:1 ratio of Triladyl®, distilled water and egg yolk, respectively, in an equivalent to 20% of its final volume. The stock solution is prepared by pouring 20% Triladyl® concentrate into a graduated flask, adding 60% sterile pure water. To complete the diluent, 20% fresh egg yolk must be added to the stock solution, maintaining the diluent ratio (1:3:1) and the final solution filtered through a sterile filter-funnel. 12 ml of diluent was prepared, taking 4.2 ml of Triladyl®, 7.2 ml of bi-distilled water and 4.2 ml of egg yolk. The dilution was filtered using the filter of a seminal collection bag, obtaining 6 ml of total dilution. The pH of 6.76 was immediately measured and the dilution was kept in a test tube at 37°C in a water bath.

Table 5. Chemical composition of Triladyl® diluent per 100 ml of prepared diluent

Item	Quantity (mg/100ml)
Tylosin	5,7
Gentamicin	28,6
Spectinomycin	34,3
Lincomycin	17,2

The dilution of the fresh semen samples was carried out following the pre-dilution methodology in a 1:1 ratio, applying 0.46 ml of semen and 0.46 ml of extender, in a water bath at more than 37 ºC. At the time of dilution, the diluent was at the same temperature as the ejaculate (+/-1 ºC). With the ejaculate evaluated, the final dilution was made, homogenizing the dilution at 37 °C in a water bath and packaging in 0.5 ml straws with a concentration of 100 x 106 Spz/dose. Subsequently, the diluted semen was packaged and frozen for future studies on cryopreservation of male alpacas' semen.

Macroscopic and microscopic characteristics of semen

The macroscopic characteristics of male alpacas' semen in the two experimental groups were determined according to the experimental procedures of Vivanco (2002). The total ejaculate was taken, without the pre-sperm fraction, and the ejaculate volume was measured using a direct reading graduated tube in milliliters. The pH was immediately measured with a digital pH-meter (pH-meter PCE-228 with pH electrode PE 03, Spain). A range between 6.20 and 6.80 was considered as normal semen pH. Color was evaluated by direct visualization of the ejaculate in a collection tube based on white scales and a numerical range of 1, 2, and 3 as reference shades (creamy white, milky white, and transparent or translucent, respectively). On the other hand, the odor as a subjective measure was measured considering normal when its odor was neutral or not unpleasant. Therefore, the perception was made directly from the collecting tube after ejaculation, a numerical range of 1, 2 and 3 as normal odor, urine odor, unpleasant odor, respectively.

The microscopic analysis was evaluated following Vivanco's recommendations (2002). Sperm concentration (x 106/ml) was determined using a Neubauer chamber, with a dilution of 1:400; In an Eppendorf tube, 5 µl of seminal sample was pipetted into 2 ml of 1% formolded physiological solution, homogenized and pipetted into the Neubauer chamber. After 5 min of sedimentation, the number of spermatozoa was counted using a 40X optical microscope (OLIMPUS BX53 Microscope).

The mass motility assessment was performed subjectively in fresh semen, with this test the spermatozoa movement was evaluated as a group. 5 µL of semen were pipetted into an object slide on a thermal stage under the microscope at 37 °C, with 100X magnification. The assessment was made using a scale from 0 to 100%, where: the formation of extremely high waves was greater than 90%. Waves or eddies are formed quickly between 70 - 90%, presence of waves or slow eddies between 50 - 70%, presence of motile sperm, but in insufficient quantity to form waves between 20 - 50%. Some spermatozoa move in place between 1 – 20%, no motile spermatozoa 0%.

The percentage of individual motility was determined by pipetting 5 µL of semen diluted in an isotonic 2.92% NaCl solution onto a slide at 37 °C, observing the reading under an optical microscope at 400X magnification. Individual motility was evaluated on a scale from 1 to 5, where: 1 refers to poor motility between 0 and 20%, 2 poor motilities between 20 and 40%, 3 medium motility between 60 and 80%, 4 for good motility between 60 and 80% and 5 for a very good motility valued between 80 and 100%. In addition, those ejaculates with progressive individual motility ≥ 70% (those found within group 3 on the scoring scale) were considered normal.

The percentage of abnormalities was determined as described by Felicidad et al (2016). They determine primary abnormalities such as spermatozoa presenting giant head, microcephalic, coiled tails, broken tails, double heads, double tail, tails around the head of the spermatozoon. Secondary abnormalities are sperm with loose or single head, single tail, hooked tail, bent tail.

The sperm viability percentage was determined by the Eosin – Nigrosin staining method. 10 µL of the dye were pipetted onto 10 µL of the homogenized pure semen sample on a slide at 37 °C, allowed to settle for 1 min, and a smear was made. The sample was observed under a microscope at 100X, counting a minimum of 100 spermatozoa in different fields of the slide. Sperm with a red or dark pink head are considered dead, while sperm with a white or light pink head are considered alive.

Statistical calculations and analysis

The microscopic analysis was evaluated following the quadrants observed from the corners to the center of the microscope and its count was made by taking 5 squares from each of the observed grids, the average was used in the mathematical equation proposed by Mayorga (2016):

Concentration (x 10^6 / ml) = X * Fd * Ac * Fc

Where:

X: Average

Fd: Dilution factor

Ac: Neubauer chamber height

Fc: Concentration factor

The percentage of abnormalities was determined as described by Felicidad, et al (2016), the following mathematical expression was used to determine the percentage of sperm abnormalities:

$$\% \text{ Abnormal Sperm} = \frac{\text{Nº Abnormal sperm}}{\text{Nº Counted sperm}} \text{ x } 100$$

The percentage of sperm viability was calculated by subjective determination using the following formula according to Felicidad et al. (2016):

$$\% \text{ VE} = \frac{\text{Nº Number of Live Sperm}}{\text{Nº Counted Sperm (Alive+Dead)}} \text{ x } 100 \qquad (3)$$

Trace element inclusion data on semen characteristics were analyzed with a one-way ANOVA using the GLM procedure from SAS, Institute Inc. (2019). The conservation data of the seminal material were independently analyzed for the inclusion of diluents and trace elements with a factorial model using the PROC MIXED from SAS, Institute Inc. (2019). The effects of treatment, diluents, and treatment × diluents interaction was considered fixed and the animal effect was considered random. In all the experiments, the values with $P < 0.05$ were considered significant, in addition, the values with $P < 0.10$ were considered a trend.

RESULTS AND DISCUSSION

Macroscopic and microscopic features

The macroscopic and microscopic characteristics between semen samples from male alpacas with trace element fixation (SCO) and without trace elements (SSO) are shown in Table 6. The volume ($P = 0.357$) collected and used in both groups was similar at 1.21 and 1.31 mL for the SCO and SSO group, respectively. On the other hand, the pH, color, and odor of the semen did not show significant differences when the trace elements were applied compared to the samples without trace elements ($P = 0.125$ and $P = 0.444$, respectively). This is possibly due to the inclusion of Thoromangan® as a source of direct trace elements in the semen of animals with a neutral pH and non-offensive odors (it does not alter the semen of male alpacas' pH). In addition, the amount incorporated (5 mL) in the SCO samples does not cause macroscopic alterations in the seminal material (Vivanco, 2002). Likewise, Garnica et al. (1993) and Bravo et al. (1998), applied similar amounts of trace elements to the alpacas' semen to evaluate the macroscopic characteristics of the seminal material before artificial insemination. They affirm that trace elements infusion in the semen does not alter the pH (average value: 7.4), nor the odor and color of the semen, which is generally described as milky or creamy white depending on the sperm concentration, with neutral pH and not unpleasant odor (Vaughan et al., 2003).

The microscopic characteristics shown in Table 6 show the effect of treating the seminal material with trace elements (Thoromangan®) versus not treating it with trace elements (SCO and SSO, respectively). No significant difference was observed in mass motility or progressive motility for both groups (SCO and SSO) with 36% and 2.01% on average respectively. Furthermore, vitality ($P = 0.118$)

and abnormalities (P = 0.118) were similar for both groups, with vitalities ranging from 89.9% for the SSO group and 90.2% for the SCO group. The percentage of abnormalities range from 9.8% and 10% for the SCO and SSO groups, respectively. On the other hand, in a sperm volume of 1.21 and 1.31 ml for the SCO and SSO groups, respectively, similar sperm concentrations were obtained (on average 46.3% and P = 0.289). Demonstrating that treating the seminal samples of male alpacas with trace elements does not alter the microscopic characteristics of the semen. These values are similar to those obtained by Bravo et al. (2000) and Vivanco (2020) in semen of male alpacas. However, a study carried out by Vaughan et al. (2003), observed that the inclusion of enzymes and trace elements in the semen of male alpacas improved sperm vitality and concentration, as well as post-freezing handling.

Table 6. Macroscopic and microscopic characteristics of semen from male alpacas treated (SCO) and untreated with trace elements (SSO).

Item[a]	Artificial vagina		SSE	P value
	SCO	SSO		
Macroscopic features				
Volume, ml	1,21	1,31	0,057	0,357
pH	7,245	7,371	0,077	0,125
Odor	1,1	1,3	0,087	0,454
Color	1,1	1,2	0,718	0,444
Microscopic features, %				
Mass motility	36,8	35,3	0,109	0,142
Progressive motility	2,0	2,1	0,109	0,510
Abnormalities	9,8	10,1	0,449	0,118
Vitality	90,2	89,9	0,071	0,118
Sperm concentration, EC	45,9	46,6	0,057	0,289

[a] SCO: semen of male alpacas with trace elements, SSO: semen of male alpacas without trace elements. CE: x 10 6/mL. Odor measured on a numerical scale from 1 to 3 for normal odor, urine odor, and unpleasant odor. Color measured on a numerical scale from 1 to 3 for creamy white, milky white, and transparent. [b] SSE: sample standard error (n = 21)

The duration of copulation can affect semen quality (Vivanco, 2002). In this study, copulation time (> 10 min) when a manikin is used, leads to better volumes per ejaculation. Reasonably, the total number of spermatozoa increases for a longer duration of copulation, since the dummy provides greater susceptibility to copulation, on the contrary, it does not exist with the female (Vaughan et al., 2003). On the other hand, semen quality can also be affected when the environment inside the artificial vagina is not controlled (Vivanco, 2002; Aller, 2003). It may reduce semen quality during prolonged intercourse, possibly when the temperature of the artificial vagina decreases over time, however, in this study, prolonged time did not decrease sperm quality. Similarly, when the semen remains in prolonged contact with the latex coating it can be detrimental to sperm survival (Aller, 2003). However, the SCO and SSO semen were not affected by the seminal collection method and show a sperm concentration of 45.9 and 46.6 %, respectively, in an average volume of 1.3 mL.

Fresh semen storage

The semen of male alpacas is difficult to handle in the laboratory due to its high viscosity. Therefore, sperm cell preservation is based on the use of diluents to provide adequate nutrients that neutralize the pH changes produced by the metabolism of sugars and protect spermatozoa from the drop in temperature during the freezing process. (Vaughan et al., 2003). However, several of the diluents commonly used for the preservation of ruminant semen have been applied to alpaca and llama semen

such as Triladyl® and AndroMed®, showing good results for cryopreservation. (Raymundo et al., 2000; Vaughan et al., 2003; Bravo et al., 2013).

The seminal samples were prepared and subjected to a dilution procedure with two commercial diluents Triladyl® and AndroMed®, and an experimental diluent (Tris-Yolk + Glycerol), were used for the preservation of male alpacas' seminal material. The evaluation of the macroscopic and microscopic characteristics of refrigerated semen as well as those treated with trace elements and untreated are shown in Table 7. The macroscopic characteristics did not show significant differences between SCO and SSO treatments, nor the use of diluents Triladyl®, AndroMed® and Tris-Yolk + Glycerol, nor the interaction between treatment and diluent ($P > 0.05$).

The pH of male alpacas' seminal content with the inclusion of the diluents, SCO and SSO showed a pH of 7.227 and 7.305, respectively (TRT: $P = 0.571$, diluent: $P = 0.999$ and TRT * diluent: $P = 0.563$). Neutral pH values and in agreement with those found in other studies that used the same diluents for the preservation of male alpacas' semen (Raymundo et al., 2000; Vivanco 2002). The infusion of diluents Triladyl®, AndroMed®, and Tris-Yolk + Glycerol did not alter the odor in fresh seminal samples ($P = 0.878$). At the same time, the interaction between treatment and diluent was not affected ($P = 0.878$), retaining the normal characteristic semen odor in all three groups. In addition, the inclusion of diluents also did not affect creamy white semen color over the TRT, diluent, and TRT * extender interaction ($P = 0.456$, 0.571, and 0.751, respectively). The color retained its normal characteristic, peculiar to camelid species; Vaughan et al. (2003). They show studies on semen collection from male alpacas and camelid species such as llamas and camels for cryogenic preservation of their seminal material and subsequent artificial insemination. Exposing alpacas' and llamas' semen with creamy white or white colorations and normal characteristic odors to the semen of South American camelids.

On the other hand, microscopic characteristics did not show significant differences between SCO and SSO treatments, nor the use of diluents Triladyl®, AndroMed®, and Tris-Yolk + Glycerol, nor the interaction between treatment and diluent ($P > 0.05$, Table 7). Mass motility, when Triladyl® was used on the SCO and SSO group was 38.4% and 34.3% respectively, in the same way when AndroMed® was used (SCO: 36.45 and SSO: 36.6%). and Tris Yolk + Glycerol (SCO: 35.6% and SSO: 36.6%). However, a trend ($P = 0.081$) was observed towards a higher percentage of mass motility when trace elements were incorporated into the seminal material, on the interaction between the treatment and diluent. In a study conducted by Vaughan et al. (2003), the infusion of trace elements in fresh alpacas' semen was evaluated and observed that the infusion maintains a good percentage of mass motility in male alpacas' semen. Likewise, Bravo et al. (2013), states that the percentage of mass motility is not affected by the number of ejaculations of the same male, motility between 49% and 70% was found, similar to those observed in this work.

Abnormalities were 9.6% on average for the research groups with the use of trace elements to improve their sperm condition. It was not affected by TRT, nor by the inclusion of diluents, nor by the treatment and diluent interaction ($P = 0.162$, 0.671 and 0.839, respectively). At the same time, sperm vitality and concentration ($P > 0.05$; mean value: 90.4% and 49.1%, respectively) were similar for the research groups. However, sperm viability, motility, and concentration of semen after dilution vary between evaluations and few studies have compared the diluents effect (Triladyl® and AndroMed®) in alpacas. In addition, in trials (Bravo et al., 1998; Bravo et al., 2013) where Tris-buffered diluents are used, egg yolk and skimmed milk show sperm vitality around 36%, 22%, and 16%, respectively. lower values than those presented in this study with sperm vitality greater than 90% when Triladyl®, AndroMed® and Tris Yolk + Glycerol were used.

CONCLUSIONS

The use of the dummy and artificial vagina in the semen extraction process guaranteed a successful extraction and despite there being a trend in the assessment of the results obtained in the characteristics of fresh semen, there was no difference in the evaluated parameters. The semen

conservation process analyzed compared to the application of trace elements does not have a difference, despite heterogeneous research on semen conservation. It is recommended to make more scientific contributions to the infusion of trace elements and diluents into fresh male alpacas' semen

Table 7. Evaluation of the macroscopic and microscopic characteristics of fresh semen from untreated male alpacas (SSO) and treated with trace elements (SCO) under refrigeration.

Item[a]	Triladyl		AndroMed		Tris Yolk + Glycerol		SSE	P value		
	CON	SIN	CON	SIN	CON	SIN		TRT	Diluent	TRT x Diluent
Macroscopic features										
pH	7,283	7,251	7,227	7,305	7,254	7,278	0,051	0,571	0,999	0,563
Odor	1,3	1,1	1,0	1,1	1,3	1,3	0,161	0,476	0,878	0,878
Color	1,1	1,1	1,1	1,4	1,1	1,1	0,154	0,456	0,571	0,751
Microscopic features, %										
Mass motility	38,4	34,3	36,4	36,6	35,6	36,6	1,180	0,307	0,934	0,081
Progressive motility	1,9	2,1	2,1	2,0	1,9	2,0	0,121	0,484	0,678	0,224
Abnormalities	9,6	9,6	9,6	9,6	9,6	9,6	0,037	0,162	0,671	0,839
Vitality	90,3	90,3	90,5	90,4	90,4	90,3	0,094	0,623	0,492	0,929
Sperm concentration, CE	49,1	48,9	49,0	49,2	49,1	49,1	0,542	0,983	0,986	0,919

[a] CON: semen of male alpacas with trace elements, SIN: semen of male alpacas without trace elements. Odor measured on a numerical scale from 1 to 3 for normal odor, urine odor, and unpleasant odor. Color measured on a numerical scale from 1 to 3 for creamy white, milky white, and transparent. CE: x 10^6/mL. [b] SSE: sample standard error (n = 7)

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