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Alpaca semen characteristics previous to a mating period

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Abstract

Increasing the knowledge of the semen characteristics in the alpaca will contribute to understanding one of the many factors that affect the poor fertility rate in this species. Ten adult male alpacas, 2.6–10 years of age, average weight 64.7 ± 4.7 kg were used. The animals were distributed randomly into two groups of five each and submitted alternatively to two semen collections, using an artificial vagina and sexually receptive females. For the first semen collection the animals had a sexual rest period of about 90 and 45 days before the second. Duration of semen collection, color and volume of ejaculate were recorded, and sperm concentration and morphology (light microscopy) were evaluated. Descriptive statistical analyses were used for each variable, considering all samples obtained ($n = 19$). An analysis of variance for animal groups and opportunity of collection were used for quantitative variables. Most frequent color was opalescent white (84.2%). There were no statistical differences among male groups or between semen collections. The average values and standard deviations for the quantitative variables were: 12.3 ± 7.2 min for semen collection time, 1.8 ± 0.8 ml for ejaculate volume, $(17.6 \pm 26.1) \times 10^6$ sperm/ml for sperm concentration and $34.0 \pm 52.2 \times 10^6$ for total number of sperm per ejaculate. The percentage of normal spermatozoa was 51.0 ± 12.4%. From the total abnormalities, that of mid piece segment (14.4%) was the most frequent. These results indicate that male alpaca have poor semen quality, when compared with other domestic species. Nevertheless, for the evaluation of male alpaca as breeders it would be necessary to create a protocol for the selection of them, where phenotypic, behavioral and seminogram aspects are considered. The values reported herein define the characteristics of the alpaca semen that

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could be considered as the initial base of the seminal analysis to select male alpacas before mating.

Keywords: Alpaca; Semen; Spermatozoa; Reproduction; Mating

1. Introduction

Many aspects of the reproductive physiology of South American camelids (SAC), mainly of males, have still not been studied (Fernández-Baca, 1993; Brown, 2000). In general, alpacas have poor fertility, with pregnancy percentage between 40 and 60% (Quispe, 1996). These values could be explained either by reproductive failures of females or males. According to Ax et al. (2000), the most desirable method to determine the fertility of males, besides their capacity to mate is to evaluate the characteristics of their semen.

A tendency has been observed for greater sexual activity during the summer months (Pollard et al., 1995). However, the management of the mating system easily modifies this situation, taking advantage of the induced ovulation that characterizes alpacas.

Both practice and research have demonstrated that several mating systems exist. Any of them can be used, depending on the criteria established for the mating process, such as the number of alpaca males available, the herd size to breed and the personnel required for management (Quispe, 1996). All systems include periods of sexual rest, even the simplest one, continuous mating, consisting of maintaining males and females together during the whole year. Mating can occur at any time, however, decreased sexual activity is observed in the periods of lesser forage availability. When the encounter of males and females is allowed during a limited mating period, males show an intense sexual activity in the first and the second day. According to Smith et al. (1994), approximately 70% of the females are mounted in this period. However, the sexual activity decreases thereafter (Fernández-Baca, 1971).

There is great variability in the characteristics of the semen of alpaca. Bravo et al. (1997a) indicate that this variation could be related to sexual activity of the male.

Different reports indicate characteristics of the alpaca semen, such as appearance, color, pH, ejaculate volume, sperm concentration and morphology (Sumar, 1991; Bravo et al., 1997a; Urquieta, 1998). However, previous reports do not indicate the sexual activity of males before sampling. The lack of this information makes for difficulty in establishing reference values for the seminogram characteristic of this species. The present study seeks to characterize alpaca semen obtained at the onset of a mating period after a sexual rest of at least 45 days.

2. Material and methods

The study was conducted in “Hidango” Experimental Centre (EC), that is a part of the Institute for Agricultural Research (INIA) of the Ministry of Agriculture, located in Chile (34°6′S; 71°45′W, 250 m altitude). From a herd of 31 alpaca males (Huacaya) with proven sexual experience, 10 were randomly selected and assembled in two groups (A and B) of five animals each. Two groups were used to minimize the possible seasonal effects, to

produce the least interference with the breeding schedule of Hidango EC and this provided a way to manage the animals so to facilitate the collection and processing of the samples. Each group was sampled on two different occasions according to the EC mating program, which consists of four mating periods starting at the onset of each season. The mating period duration was of 12 days with two service phases of 5 and a 2 days rest in between (days 6 and 7). The sampling of semen was conducted on the first day of the spring and summer mating periods, and between these dates. The males were between 2.6 and 10 years of age with an average body weight (±S.D.) of 64 ± 4.7 kg. Two semen collections from all males were attempted. Males had a sexual rest of at least 90 days before the first semen collection and 45 days for the second sampling.

The semen collection was conducted by using an artificial vagina (AV) and receptive non-pregnant females. The AV was made of a PVC cylinder 4 cm in diameter and 20 cm in length. The inside of the cylinder was lined with a natural latex tube and sealed to the PVC tube at both ends by means of elastic rubber bands, leaving a cavity at the center. Through a valve attached to the cylinder, the compartment left between the latex and the PVC tubes was filled with warm water (40 °C) with a disposable syringe. Externally the AV was covered with high-density plastic foam as thermal insulator. Inside the AV, a polyethylene bag 8 cm wide and 30 cm long was placed to receive the semen sample (Urquieta, 1998; Flores, 2001). The temperature of the AV was maintained at the alpaca body temperature (39 ± 0.5 °C) (Parraguez et al., 1993) during the sample collection. Once the sample was obtained the semen was maintained at 38–39 °C in a thermos flask for a period that did not exceed 2 h before evaluation.

Each male was presented to two or three females. The courtship phase was allowed and at the time when the male and female adopted the sexual mating position, the penis was diverted towards the AV to obtain the ejaculate. Because the ejaculation of alpaca is a continuous emission of semen after penetration, the duration of ejaculation was measured (in minutes) from the introduction of the penis into the AV until the male voluntarily remove the penis from the AV. This time was registered as the duration of semen sample collection and does not include those periods when the penis was outside the AV during ejaculation.

The ejaculate was emptied into graduate conical tubes to determine the volume (ml) and the subjective evaluation of color, which was classified in opalescent white, white, yellowish white and colorless translucent (Flores, 2001). An aliquot of each ejaculate was obtained for duplicate smear preparation for morphological evaluation. Another aliquot was diluted in a solution (1:1 v/v) for sperm concentration determination (WHO, 1999). The samples were kept refrigerated (4 °C) until laboratory processing. The following measurement or observations were made:

- Sperm concentration, the number of spermatozoa per milliliter, was determined using a hemocytometer (WHO, 1999).
- Total spermatozoa per ejaculate, number of spermatozoa per ejaculate was calculated from the volume and sperm concentration.
- Sperm morphology, was evaluated from smears stained with Harris' haematoxylin and eosin (WHO, 1999) by light microscopy. Loose heads were counted separately.

A descriptive statistical analysis was performed for each variable, the average and the standard deviation was obtained. The results of quantitative variables: time of semen

collection, ejaculate volume, sperm concentration and total number of spermatozoa per ejaculate were compared with an analysis of variance (ANOVA), organized in a completely randomized design, considering the groups of animals as the replicates and the opportunities of semen collection as treatments. Finally, those variables that were expressed as a percentage such as sperm morphology were normalized by angular transformation (Little and Hills, 1978) to conduct an analysis of variance.

3. Results

3.1. Consistence and color of the semen

The samples appeared frothy immediately after collection. This characteristic disappeared as time passed (2 h). After this period the semen had a consistency similar to a thick mucous liquid. The color of the semen was predominantly opalescent white; present in 84.2% of the samples. The rest of the semen was either white (10.5%) or yellowish white (5.3%).

3.2. Time of sample collection, semen volume, sperm concentration and total number of sperms per ejaculate

The analysis of variance that was performed to evaluate these variables indicated that there were no statistically significant differences, among the groups of males or among semen collections. To obtain the overall averages of each one of the variables the total number of observations was considered. These values are presented in Table 1.

3.3. Sperm morphology

Out of the 18 samples studied by means of light microscopy there were only 16 of them (88.9%) where it was possible to complete the study of the spermatozoa morphology. No spermatozoa or a very reduced number of them were observed in the smears of two samples (11.1%).

The percentages of normal and abnormal spermatozoa were not different for group of males or collection of semen time. The average percentage (±S.D.) of normal and abnormal

Table 1
Values observed for duration of semen collection (min), volume of semen (ml), sperm concentration (sperm × 10^6/ml) and total number of spermatozoa per ejaculate (sperm × 10^6)[a]

Variable	*n*	Mean ± S.D.	Max–min
Duration of semen collection (min)	19[b]	12.3 ± 7.2	28.3–2.50
Volume of semen (ml)	19[b]	1.8 ± 0.8	3.8–0.60
Sperm concentration (sperm × 10^6/ml)	18[c]	17.6 ± 26.1	92.9–0.05
Total sperms per ejaculate (sperm × 10^6)	18[c]	34.0 ± 52.2	167.2–0.06

[a] Number of samples (*n*), mean ± S.D. and maximum and minimum.
[b] During the first semen collection one male rejected the AV.
[c] One of the 19 samples was azoospermic.

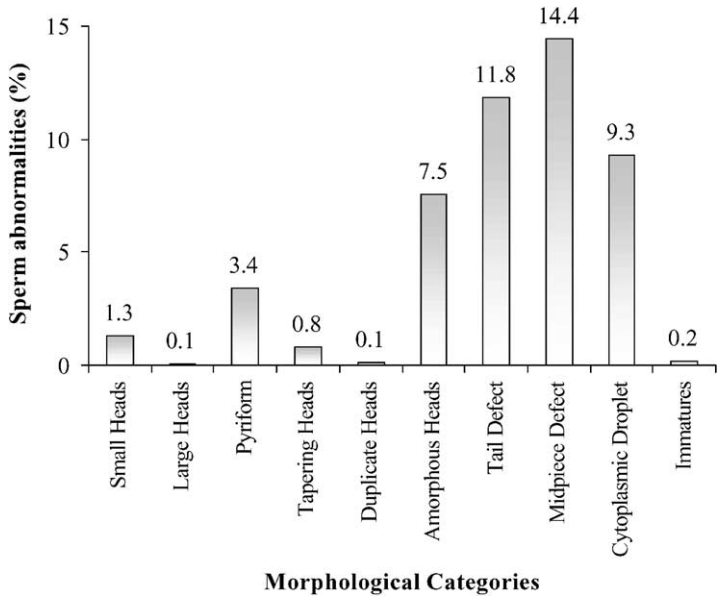

Fig. 1. Distribution of sperm morphological abnormal categories in alpaca semen. The total percentage of abnormal spermatozoa was 49%.

spermatozoa was 51 ± 12.4 and 49 ± 12.4%, respectively. The most frequent abnormality corresponded to the mid piece defect (14.4%), followed by those of the head (13.2%) and thirdly those of the tail (11.8%). The percentage obtained for each morphological category of abnormality is depicted in Fig. 1.

Loose heads, although observed in the smears, is a form of spermatozoa that was not included in the sperm morphology classification. However, this type of spermatozoa appeared in 87.5% of the samples. A mean value for this variable of 14.8% (±15.2) was found with a range between 3.0 and 62.5% with the percentages calculated as a base of the spermatozoa that were classified. The percentage of loose heads after at least 90 days of sexual rest or a rest period of 45 days was 19.3 ± 21.2 and 10.9 ± 6.8, respectively. These values were not statistically different as indicated by the analysis of variance with the arcsine transformation of the data.

4. Discussion

Among the physical characteristics of the ejaculate immediately after collection, a frothy consistence was observed. This observation is consistent with previously conducted studies in alpaca (Ferré et al., 1996; Urquieta, 1998) and could be related with the material (polyethylene) used to receive the ejaculate as internal liner of the AV. Nevertheless, most reports have indicated the prevalence of highly viscous ejaculates that are difficult to manipulate.

The color of the semen in the present study was similar to that previously reported, independent of the technique used for semen collection (Garnica et al., 1993; Bravo et al., 1997a,b; Urquieta, 1998).

The duration of semen collection of 12.3 min on average, which was similar with the duration reported for natural mating (Bravo et al., 1997c). However, it was between 3 and 5 min shorter, than that reported in other studies when using an AV and an artificial device (dummy) for males to mount (Bravo et al., 1997a,b; Davalos et al., 1999). It could be assumed that the duration of mating observed in the present study, compares favorably with data where there was natural mating in previous studies and can be used as the sampling period to evaluate the semen characteristics of the alpaca. The volume of semen collected was 1.8 ml. This value is slightly greater as compared with those previously reported by Garnica et al. (1993), Bravo et al. (1997a) and Davalos et al. (1999), when using an AV and dummy; and by Urquieta (1998) and Davalos et al. (1999), when using an AV and receptive females for males to mount during semen collection.

The sperm concentration at the onset of a mating period (17.6×10^6 sperm/ml), was less as compared with the value indicated by Bravo et al. (1997a) (56.2×10^6 sperm/ml), and greater as compared with that observed by Davalos et al. (1999) (3.3×10^6 sperm/ml) where in both studies an AV and a dummy were used. However, when comparing the results obtained in the present study with those previously reported where an AV and receptive females were used, such as Urquieta (1998) (8.3×10^6 sperm/ml) and Davalos et al. (1999) (5.8×10^6 sperm/ml), sperm concentrations were greater. The great variability observed for this characteristic, has also been reported in previous studies conducted in the alpaca (Bravo et al., 2000).

The average of the total number of spermatozoa per ejaculate obtained (34×10^6), was greater as compared with that observed by Bravo et al. (1997b) (2×10^6), but less as compared with that reported by the same group (75.3×10^6) in another study (Bravo et al., 1997a). An AV and a dummy were used in both of the previous studies. If the results reported in the present paper are compared with those where an AV and a receptive female were used, it is observed that the values for total number of spermatozoa are greater as compared with those found by Urquieta (1998) (18.2×10^6) and by Davalos et al. (1999) (10×10^6). The large variability of the sperm concentration and the total number of spermatozoa indicate that this condition seems to be inherent to the species.

The average percentage of normal spermatozoa found in the present study (51%) was less as compared with that reported by Bravo et al. (1997a,c) where averages of normal sperm of 73.7 and 75.9% were obtained, respectively. Also, average normal spermatozoa was less as compared with those reported by Urquieta (1998) (68.6%) and Davalos et al. (1999) (86.1%). The presence of a large percentage of abnormalities found in the present study was consistent with situations where there is storage of spermatozoa in the epididymis for long periods before ejaculation occurs (Garner and Hafez, 2000). This could be the result when there have been prolonged periods of sexual rest of at least 45 days before ejaculation. When comparing the values of sperm abnormalities with those observed in previous studies (Bravo et al., 1997a,b), it is observed that in the present study values were greater for each category of sperm abnormality. However, previous studies did not include in classification of sperm morphology, the abnormalities of the mid piece segment, which had the greatest frequency of abnormal observation in the present study (14.4%). More research is, therefore,

needed to establish with certainty the ideal length of sexual rest before the time ejaculates are collected.

Studies with electron transmission microscopy where spermatozoa of the alpaca were evaluated (Urquieta, 1998) indicated fragility of the head–neck piece insert. This observation could explain the presence of loose heads. Furthermore, the percentage of loose heads found after to 90-day rest before the period of ejaculate collection resulted in a doubling of this defect as compared with a 45-day rest period before collection. This difference in numbers of this type of abnormality between the two sexual rest periods was not statistically significant because of the variability for this variable which could be as well a result of the length of the sexual rest.

Although the animals used in the present study varied in age, this factor did not have a significant effect on any of the variables.

5. Conclusions

The seminal characteristics of the alpaca either after 45 or 90 days of sexual rest are similar. This period of sexual rest before collection of the ejaculate might be too long for the alpaca male and could explain the large percentage of sperm abnormalities found in the present study. However, more research is needed to establish with certainty the ideal length of sexual rest before collection of ejaculates in the alpaca. One of the most representative characteristics in the good performance of a male as breeder is the sperm concentration of its semen. Considering this factor, the male alpaca appears inferior when compared with other species. However, it would be necessary to define the minimum volume and sperm concentration needed in males to achieve acceptable fertility rates in this species or to design mating systems that take advantage of the male's potential with a sexual activity that does not deplete its seminal reserves. An approach for the evaluation of male alpaca breeders would be to create a protocol for the selection of them, where phenotypic, behavioral and seminogram aspects were considered.

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