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Alpaca semen characteristics under free and directed mounts during a mating period

Bessie Urquieta [a,*], Paloma Flores [a], Camila Muñoz [b], Eduardo Bustos-Obregón [c], Jorge García-Huidobro [b]

[a] *Depto. de Ciencias Biológicas Animales, Facultad de Ciencias Veterinarias y Pecuarias, Universidad de Chile, Casilla 2, Correo 15, Santiago, Chile*

[b] *Centro Experimental Hidango, CRI Rayentue, Instituto de Investigaciones Agropecuarias, Correo Litueche, Casilla 3, Litueche, VI Región, Chile*

[c] *Laboratorio de Biología de la Reproducción, Programa de Morfología, Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad de Chile, Independencia 1027, Santiago, Chile*

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Abstract

Most studies in alpaca reproductive biology have been focused on female physiology. Only recent research is being conducted in order to increase the knowledge on males. Semen characteristics during breeding periods will contribute to understanding the poor fertility rates in alpaca.

Ten adult male alpacas were distributed randomly into two groups and submitted alternatively to two regimens of semen collection of 12 days duration (day 1, initial day of semen collection). Semen samples were collected using an artificial vagina and a receptive, non-pregnant female. With regimen 1, males were maintained with females except for the days of sexual rest (6 and 7). Semen was collected on days 1, 5, 8 and 12. With regimen 2, males were exposed to females for daily semen collection only, before and after sexual rest. Mating duration, color and volume of ejaculates, spermatozoa concentration and morphology were evaluated.

No statistical differences for the variables were found between regimens that were used for semen collection. With respect to influence of day, however, the total numbers of spermatozoa ejaculated on days 1 and 5 of semen collection were statistically different ($p < 0.05$). Azoospermic samples increased on days 5 and 12 of semen collection. Partial recovery in spermatozoa concentration and number of spermatozoa ejaculated were observed after sexual rest. Although normal spermatozoa

* Corresponding author. Tel.: +56 2 678 55 79; fax: +56 2 678 55 26.
E-mail address: burquiet@uchile.cl (B. Urquieta).

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percentage was less on day 1 ($p < 0.05$) as compared with values found in the following ejaculates (days 5 and 12), the total number of normal spermatozoa was greater.

These results support the conclusion that when male alpaca have a daily ejaculation during five consecutive days, they might copulate without having enough spermatozoa for fertilization towards the end of the mating period.

Keywords: Alpaca; Semen; Spermatozoa morphology; Reproduction; Mating period

1. Introduction

Raising South American domestic camelids has been a traditional activity of the Andean highland inhabitants (Fernández-Baca, 1971). Most of the handling practices, including the reproduction aspects, have been developed for those environmental conditions where a seasonal reproductive pattern is observed.

The introduction of these species to other countries (Fernández-Baca, 1991; Brown, 2000) and their redistribution in Chile (Castellaro and García-Huidobro, 1991; Castellaro et al., 1998) have resulted in the need to develop different management considerations to improve fertility in these animals.

The alpaca (*Lama pacos*), outside their natural habitat, is considered a non-seasonal breeder, and are an induced ovulating species (Sumar, 1999). This condition allows adult non-pregnant females to mate and for males to impregnate females at any time of the year. Urquieta (1998) demonstrated that there are no significant variations between alpaca semen characteristics during winter or summer collections in males maintained in the central zone of Chile.

After ovulation is induced during copulation and if no pregnancy ensues, there are approximately 12 subsequent days when the female will not be receptive to the male. This physiological pattern results as a consequence of the lifespan of the corpus luteum when this sequence of events ensues (Fernández-Baca, 1971). This interval in females that do not become pregnant after mating leads to a lessened in efficiency of a breeding system, if males are subfertile when they mate with females.

Diverse breeding systems exist for alpaca (Quispe, 1996). Some of them have been designed without any biological base or previous knowledge about the reproductive capacity of males. Sexual activity of the alpaca male is very intense at the beginning of the breeding period, showing a pattern where there is a much greater frequency of mounting as compared to later in the breeding period (Fernández-Baca and Novoa, 1968). Bravo et al. (1997a) reported that the mating frequency modifies some characteristics of the alpaca semen, diminishing the reproductive yield of males (Bravo et al., 1997c). Therefore, the mount frequency and the total breeding period duration could influence the fertility levels of the herd.

To examine the fertility of sires, the evaluation of semen becomes a helpful alternative tool (Ax et al., 2000). In alpaca, the collection of semen has been difficult, due to the position that the animals adopt during copulation and the relatively long duration of this process in this species compared with many others (Bravo et al., 2000). However, semen collection

by means of an artificial vagina (AV) has been successful in many previous studies (Sumar and Leyva, 1981; Bravo et al., 1997b; Urquieta, 1998; Flores, 2001).

The aim of the present study was to evaluate the effect of daily semen collections on its characteristics, and to compare them with ejaculates obtained periodically in a free mounting regimen during a mating period. This knowledge could contribute to the design of breeding systems for alpacas based on seminal characteristics of sires.

2. Material and methods

The present study was conducted at the "Hidango" Experimental Centre (EC), Institute of Agricultural Research (INIA), Ministry of Agriculture, Chile, located at 34°6′S; 71°45′W; 250 m altitude. The area is characterized by a sub-humid Mediterranean climate, with a mean annual temperature of 13.6 °C and a mean annual rainfall of 897 mm, with July being the wettest month. The dry season typically extends from November through March (Novoa et al., 1989).

The alpacas were grouped by sex and age, except for during the mating periods in different natural pastures (mainly grasses), which were adequate to satisfy the nutritional needs of the herd. There was an ample supply of water in each field.

The present study was conducted between the spring and summer mating periods of 2001. Ten alpaca males (Huacaya), between 2.6 and 10 years of age, with an average weight (±S.D.) of 64 (±4.7) kg were selected at random from the breeding stock. They were distributed into two groups (A and B) of five males each for handling reasons, and to avoid interference with the normal activities at the centre. These animals were part of the breeding program used in Hidango EC. This annual program consists of four mating periods starting at the onset of each season. Each mating period lasts 12 days (day 1, initial day of the mating period), with two service phases (5 days each) separated by a 2-day sexual rest (days 6 and 7). Mating occurred in small pastures of 0.5–0.8 ha, where one male remained permanently with a group of 8–10 females during the days of service.

Two regimens of semen sampling were used alternately for each group, using a switch-back design considered as replicates 1 and 2. The sampling occurred during September and October (spring) and November and December (spring–summer), respectively. Between sampling periods, males had a 45-day sexual rest (Table 1). The two regimens included the same duration for the mating period.

Table 1
Working design for the semen collection, each group was alternatively sampled with and without females in two opportunities

Replicate 1		Replicate 2	
Spring		Spring	Summer
21 September–3 October	16 October–27 October	20 November–1 December	22 December–3 January
Group A	Group B	Group A	Group B
Regimen 1	Regimen 2	Regimen 2	Regimen 1

(a) With Females

Mounting days					Sexual Rest		Mounting days				
1	2	3	4	5	6	7	8	9	10	11	12
Sample				Sample			Sample				Sample

(b) With females only for the sampling procedure

Mounting days					Sexual Rest		Mounting days				
1	2	3	4	5	6	7	8	9	10	11	12
Sample	Sample	Sample	Sample	Sample			Sample	Sample	Sample	Sample	Sample

Fig. 1. Schemes of semen sampling for two groups of male alpacas. (a) Sampling with females. (b) Sampling with females only for the sampling procedure.

Regimen 1 of semen collection was done under customary free mating conditions, as described previously. The first collection of semen was conducted at the beginning of day 1 of the mating period. The second sampling of semen was performed at the end of day 5 of the mating period (previous to the 2-day rest period). Two more samples were obtained at the beginning of the second mating phase (day 8) and at the end of the mating period (day 12). A total of four samples of semen were obtained from each male (Fig. 1a). Sexual activity during the free mating phases was not recorded.

For regimen 2 of semen collection, males were separated from females throughout the study, except for the time of semen collection. Sampling was conducted daily for five consecutive days separated by the 2 days of sexual rest. This regimen emulates a mating period, assuming a single copulation per day based on a mean value of 1.25 mounts per day for each male. This was calculated from data that were previously reported (Fernández-Baca and Novoa, 1968). A total of 10 samples of semen were taken from each male (Fig. 1b).

Semen samples were collected using an artificial vagina while males were in the presence of a sexually receptive[1] non-pregnant female (Urquieta, 1998; Flores, 2001; Flores et al., 2002). Each male was selected at random from each group and presented to two or three females one at a time during different sampling days. The males were allowed a courtship phase of sufficient time for the pairs to adopt a stable mating position before the penis was diverted toward the AV to obtain the ejaculate. The mating duration was timed, from the moment of penis introduction into the artificial vagina until the male voluntarily removed the penis from the device. Males were not trained for semen collection before the present experiment was initiated.

The ejaculate evaluation included volume (ml) and color with the latter being classified as opalescent white, white, yellowish white or colorless translucent (Flores, 2001). An aliquot of each ejaculate was obtained for duplicate smear preparation. Another aliquot was diluted (1:1, v/v) in recount solution (5%, w/v, $NaHCO_3$; 1%, v/v, formalin 37%; 1%,

[1] Receptive alpaca female would be equivalent to an estrual female in spontaneously ovulating species.

w/v, trypan blue) for spermatozoa concentration determination (WHO, 1999). The samples were kept refrigerated (4 °C) until laboratory processing. The following measurements or observations were made:

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

The experiment was arranged in a switch-back design with exchange (Li, 1969). To compare both the two regimens of semen collection and collection dates with two replicates (spring and spring–summer collections), an analysis of variance (ANOVA) for repeated measurements was applied. The results obtained for days 1, 5, 8 and 12 (dates coincident among the two schemes) were used.

Those characteristics that were expressed in percentage, such as spermatozoa morphology, were normalized through an angular transformation (Little and Hills, 1978) to perform an ANOVA using the same model described previously.

The results of qualitative variables: color and presence of azoospermic samples were analyzed using a non-parametric ANOVA. For both variables, Wilcoxon scores (rank sums) classified by day and Kruskal–Wallis test using chi-square. Additionally, azoospermic samples were classified in a contingency table for either positive or negative, and days to prove the independence of the classifying factors using a chi-square test.

3. Results

3.1. Color of semen

The predominant color of semen was opalescent white, present in 76.9% of all samples. The other ejaculates had colors that were distributed between white (5.1%), yellowish white (5.1%) and colorless translucent (12.8%). As the mating period advanced (between days 1 and 12), a tendency was observed in the percentage of colorless translucent samples to increase, while the opalescent white diminished (Fig. 2), however, these differences were not significant either for regimens or days of sampling.

3.2. Mating duration, volume of ejaculate, spermatozoa concentration, total number of spermatozoa per ejaculate

There were no differences ($p > 0.05$) between the two regimens of semen collection conducted for mating duration, volume of ejaculate, spermatozoa concentration, or total number of spermatozoa (Table 2). The duration of mating and volume of the ejaculate did not vary as days of service advanced (Table 3). There was, however, a tendency for a decrease in spermatozoa concentration between days 1 and 5 and between days 1 and 12

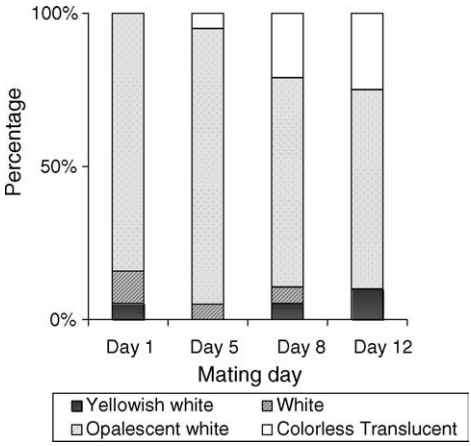

Fig. 2. Distribution of alpaca semen samples according to color category for selected dates of the mating period.

of the mating period ($p = 0.19$). Moreover, total number of spermatozoa per ejaculate was greater for day 1 as compared with day 5 of the mating period (Table 4).

For the calculation of the average values of the spermatozoa concentration and of the total number of spermatozoa ejaculated, 61 samples out of the 78 successful collections were considered; the 17 remaining samples were excluded because they were azoospermic (21.8%). These azoospermic samples, however, did not represent a permanent condition of the animals for which they appeared. The largest percentage of azoospermic samples occurred on day 5 (35.0%), followed by day 12 (30.0%) (Table 5). The contingency table provided for independence in classifying variables ($p >$ chi-square = 0.098). The Kruskal–Wallis test for

Table 2
Spermiogram for semen collection regimens—males maintained with females (1), throughout the service phases; sires with the females only during the mounting event (2) for sampling

Variables	Semen collection regimen (mean ± S.E. (n))	
	1	2
Mating duration (min)	13.10 ± 0.26 (38)	11.01 ± 1.05 (40)
Ejaculate volume (ml)	1.90 ± 0.03 (38)	1.79 ± 0.03 (40)
spermatozoa concentration ($\times 10^6$/ml)	7.79 ± 0.63 (28)	14.31 ± 0.53 (33)
Total number of spermatozoa ($\times 10^6$/ejaculate)	14.25 ± 1.11 (28)	22.09 ± 0.94 (33)

Table 3
Duration of copulation and volume of the ejaculate, means (±S.E.) of both collection regimens for days 1, 5, 8 and 12 of the mating period, (n) number of samples

Mating day	n	Duration of mating (min), mean ± S.E.	Volume of ejaculate (ml), mean ± S.E.
1	19	12.30 ± 0.37	1.78 ± 0.05
5	20	10.13 ± 0.32	2.03 ± 0.05
8	19	11.09 ± 0.34	1.88 ± 0.05
12	20	14.57 ± 0.57	1.66 ± 0.05

Table 4
Mean and S.E. of spermatozoa concentration and total spermatozoa per ejaculate of both collection regimens, for days 1, 5, 8 and 12 of the mating period, (*n*) number of samples

Mating day	*n*	Spermatozoa concentration (spermatozoa# × 10^6/ml), mean ± S.E.	Total spermatozoa per ejaculate (spermatozoa# × 10^6/ejaculate), mean ± S.E.[a]
1	18	17.60 ± 0.98 a	33.98 ± 1.73 a
5	13	6.33 ± 1.36 a	8.78 ± 2.40 b
8	16	10.71 ± 1.10 a	13.23 ± 1.95 ab
12	14	8.54 ± 1.26 a	13.60 ± 2.207 ab

[a] Different letters indicate a significant difference ($p < 0.05$) Duncan multiple range test.

Table 5
Number of zoospermic and azoospermic samples for the mating days 1, 5, 8 and 12 for both collection regimens

Mating day	Number of samples		Total
	Zoospermic	Azoospermic	
1	18	1	19
5	13	7	20
8	16	3	19
12	14	6	20
Total	61	17	78

the Wilcoxon scores for days indicated there was a tendency ($p >$ chi-square = 0.10) for a greater percentage of azoospermic samples towards the end of each mounting phase within the mating period (days 5 and 12).

3.3. Spermatozoa morphology

Out of a total of 61 samples studied by means of light microscopy, in only 48 of them (77.4%) was the complete study of spermatozoa morphology possible to assess because in the 14 remaining samples (22.6%) spermatozoa quantity was scarce. When comparing the average number of normal spermatozoa for each collection date, a significant difference was observed ($p = 0.04$) between days 1 and 5 and between days 1 and 12 with there being a lesser percentage of normal spermatozoa on day 1 (Table 6).

The distribution of abnormal forms of spermatozoa and the presence of loose heads did not vary with regimen of semen collection, or day of sampling, being similar as reported

Table 6
Mean (±S.D.) percentage of normal spermatozoa found on days 1, 5, 8, and 12 of the mating period

Mating day	Normal spermatozoa, mean percentage (±S.D.)[a]	*n*
1	51.0 (±12.4) a	16
5	59.3 (±13.5) b	11
8	56.6 (±9.9) ab	11
12	58.0 (±10.0) b	10

[a] Different letters indicate a significant difference ($p < 0.05$) Multiple range test.

previously (Flores et al., 2002). Additionally, the proportion of abnormal forms did not differ between collections in September and October and collections in November and December.

4. Discussion

The color of the semen was similar to that reported previously, independent of the technique used for its collection (Garnica et al., 1993; Bravo et al., 1997a, 1997b; Urquieta, 1998; Flores et al., 2002). For males used in regimen 2 frequency of mating was known (once daily), however, for males with females more frequent copulations could have been possible. As the duration of semen collection did not result in significant differences among the dates of semen evaluation, it could be inferred that there is no relationship between the frequency of collection of semen and the duration of copulation. Similar results were described by Bravo et al. (1997a). In another study (Bravo et al., 1997c), however, reported a sexual mating duration that was significantly shorter in males subjected to four or six mounts daily, compared with those males that mounted twice a day. In the latter study, the males had a greater number of daily mounts as compared with the present study.

The absence of differences in volume of semen among the dates of semen collection in the present study is consistent with previous reports (Bravo et al., 1997a), indicating that the ejaculate volume was not affected by frequency of mounts.

Although spermatozoa concentration was not different among dates of semen collection, a tendency for a decrease was observed ($p = 0.19$) as the mating period advanced. In most previous studies in alpaca, a great variability was reported with respect to spermatozoa concentration (Bravo et al., 2000). There can be sample values that range from those that are azoospermic to those with several million spermatozoa per ml of semen in the same animal. This variation was also observed in the present study. The great variation in spermatozoa concentrations in the present study, however, could be attributed to limitations in the use of the artificial vagina, but this method as compared with others appears to be the most reliable (Bravo et al., 2000).

The appearance of azoospermic samples in 20.5% of the total samples has also been observed in alpaca by Bravo et al. (1997a) and Urquieta (1998) who reported 13.7% and 13.3% of azoospermic samples, respectively. This finding may reflect a depletion of the spermatozoa reserves present in the epididymis (Bedford, 1990). In llama (*Lama glama*) and alpaca, it has been reported that the tail of the epididymis (main place of storage of spermatozoa for most mammals) is small in size and less marked in its morphology (Bravo and Johnson, 1994; Smith et al., 1994).

The significant decrease observed in the total number of ejaculated spermatozoa as the mating period progressed has been found previously. Bravo et al. (1997a,c) reported that when increasing the ejaculation frequency in the male alpaca, the total number of spermatozoa and fertility diminished. It is possible that when sires are subjected to a greater frequency of mounts, either daily or accumulative over consecutive days, this sexual activity will account for a decrease in the quantity of spermatozoa present in ejaculates. The males will usually continue mounting females, but their semen might not have the spermatozoa quantity and quality to result in a pregnancy. When the male remains for several days in the

presence of females in any breeding system, conpulations can induce ovulation, but without having adequate numbers of spermatozoa in the ejaculate pregnancy will not result from the matings.

A recovery was observed in the total number of spermatozoa ejaculated, however, at the end of the sexual rest in the present study. Although this increase was not statistically significant, it suggests what could happen if the sexual rest was increased if the mating system that was utilized in the present study was used. In the "alternate mating" system, males remain in service from 3 to 7 days and then are replaced with other males. This system, though designed to overcome behavioral changes in males, has resulted in increased natality rates after prolonged (93 or 60 days, respectively) mating periods (Sumar, 1991).

The increase in the average values of normal spermatozoa as the mating period advances has not been reported previously in alpaca. The males, after having a minimum of 45 days without sexual activity had a greater proportion of abnormalities in their spermatozoa than during the mating periods. This could be due to an excessive time of storage of the spermatozoa in the epididymis, losing their viability. This problem can be decreased so that the greatest spermatozoa concentration is observed in the first ejaculates following a prolonged sexual rest. Flores et al. (2002) reported that semen characteristics are similar after rest periods of 45 or 90 days, which would allow for an increased number of mating periods throughout a yearly cycle.

Other authors (Bravo et al., 1997a) reported in alpaca that the percentage of normal spermatozoa is not affected by ejaculation frequency and lesser percentage of normal sperm in the first ejaculates was not observed. However, males in this previous study were submitted to a training period (mount a dummy) and there is no indication as to the time they remained sexually inactive between the end of training and the beginning of the study.

In other species, it has been observed that ejaculates collected after a lingering sexual rest contain a greater percentage of degenerated or aged spermatozoa (Garner and Hafez, 2000). Preston et al. (2001), however, in Soay rams affirmed that as the mount frequency increased, the quantity of normal spermatozoa present in ejaculates diminished. The latter finding differs with the results of the present study.

As there were no differences among the two regimens of semen collection in the present study, the maintenance of sires together with females throughout the mating period would produce the same effect on the ejaculates as separating them after having had one mount per day. The male behavior, however, changes when they remain next to the females from several days. There is a diminished libido and compromised sexual activity throughout the day. This could be observed in some males used in regimen 1 of the present study. In a system of controlled mounting such as that conducted when regimen 2 was used in the present study, the males manifest an appropriate sexual behavior and a libido that sustained a daily sexual mating frequency. The results suggest that the frequency of mounts in the first days of the breeding period should not be excessively great as has been described in other studies (Fernández-Baca and Novoa, 1968), even considering the differences in mating systems.

In alpaca, the spermatozoa count in ejaculates are less when compared with other animal species (Flores et al., 2002). The site of semen deposition during mating is intra uterine which may reduce the need of greater volume of ejaculate and spermatozoa concentration in this species.

The decline in spermatozoa reserve capacity towards the end of each mounting phase indicates that a mating system should be structured in such a way that takes advantage of the increased concentration of spermatozoa at the initiation of the mating period. The length of the rest period used in the present study appears to be shorter than optimal and more research is needed to establish the appropriate length that allows the males to recover the initial semen characteristics without inefficient use of the male for breeding purposes.

5. Conclusions

The duration of the copulation and the ejaculate volume is not affected by one mating each day for five consecutive days.

At least one mount each day for five consecutive days results in a decreased total quantity of spermatozoa ejaculated, which could lead to a decreased fertility in herds of alpaca.

The results suggest that a mating system should consider 5 days as a maximum for the service phase. Beyond that duration of mating period, the male should have an undetermined duration of sexual rest. Further investigations are needed to establish the minimum length of rest to recover the initial number of spermatozoa per ejaculate while maintaining the least number of abnormalities. The 2-day period of sexual rest used in the present study appears insufficient for optimizing fertility of alpaca males.

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