

SPERMATOZOA CHARACTERISTICS RECOVERED FROM THE FEMALE REPRODUCTIVE TRACT AFTER COPULATION OF AN INDUCED OVULATOR, THE ALPACA

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Abstract: Alpaca's spermatozoa characteristics, recovered from the uterus and oviducts and at defined times after copulation, were determined. Uteri and oviducts from 24 females were dissected separately, and spermatozoa's motility, viability, concentration, and morphology were determined. Data were analyzed using the analysis of variance. There were changes ($P \leq 0.05$) in motility and concentration. Motility was 70.7, 37.5, 31.9, 59.3 and 63.7% for 0, 12, 24, 36, and 48 hours, respectively. Likewise, sperm concentration was 3.8 and 2 million spz/mL by 0 and 48 hours after copulation. Sperm viability and morphology did not change ($P \geq 0.05$) between the uterus and oviducts, being 96.5%. Normal spermatozoa were 97% in both reproductive organs. Decreased spermatozoa motility by 24 hours might represent a latency period, and increased motility by 36 and 48 hours is a sign of readiness for fertilization.

Keywords: Spermatozoa; Uterus; Oviduct; Alpaca.

INTRODUCTION

The reproductive events of alpacas are unique because males deposit semen into the uterine horns with continuous ejaculation starting 5 minutes after penis intromission and over a 15 to 20 minutes copulatory period (Franco et al., 1981). In addition, spermatozoa are entrapped within a thick seminal plasma, which limits the expression of its motility (Garnica et al., 1993). Spermatozoa may reach the oviducts after 6 hours, and with the utero tubal junction acting as the main reservoir up to 36 hours from copulation (Bravo, Moscoso; 1994), and it has been reported that spermatozoa are sequestered within oviduct mucosal folds awaiting ovum for fertilization (Apichela, 2008). In the rabbit, another induced ovulator, spermatozoa, reached the oviduct in two manners, one denoted as rapid

transport and present in the oviducts within minutes after insemination or copulation to a male rabbit (Suarez et al., 1983). The second is called slow transfer, with spermatozoa reaching the oviducts after several hours from copulation. In the alpaca, it is still unknown if spermatozoa maintain their characteristics inside the uterus and oviduct for up to 48 hours because ovulation is triggered by copulation and occurs within 24 to 36 hours from the time of copulation (San Martin et al., 1968). The main objective of the present study was to determine spermatozoa characteristics collected from the uterus and oviduct in females at 12 hours intervals and copulating with an intact male at time zero.

MATERIALS AND METHODS

ANIMALS

Twenty female alpacas and two males with positive reproductive histories were used in this study. They were maintained on native pastures at the La Raya research center in the Southern Peruvian highlands at 4200 m sea level, 14 °South latitude, and 70 ° West longitude.

EXPERIMENTAL DESIGN

Females were divided randomly into five groups of four each to be slaughtered at 0, 12, 24, 36, and 48 hours after a copulatory period with an intact male. Reproductive organs were rapidly dissected from females after slaughter and following guidelines from the research committee for the humane treatment of animals. Uteri and oviducts were separated and flushed with Tris extender solution. For uteri, 2 mL was used, and for oviducts, 1 mL. The washed solution was immediately kept at 35 °C until evaluation. Spermatozoa characteristics determined were motility, viability, concentration, and morphology using the CASA system (Microptic Laboratories,

Barcelone, Spain).

ANALYSIS OF DATA

Data analysis was performed using the general linear model and the Number Crunching Statistical system, Layton, UT, USA., with $P < 0.05$ considered significant. Duncan's test was used to compare means.

RESULTS

Motility and concentration varied with time and differed in the uterus and oviducts (Figures 1 and 2). The motility details of alpaca spermatozoa are summarized in Table 1. At 0 through 12 hours, spermatozoa presented their tail oscillating in place with no forward movement. Motility changed by 36 hours after being activated with forwarding head movement and its tail moving laterally (Fig. 3). Viability and morphology did not change ($P \geq 0.05$) within the uterus and oviducts throughout the study period, Table 2.

DISCUSSION

Alpaca spermatozoa motility presented two characteristics. First, motility followed the same trend in the uterus and oviducts, i.e., increased motility immediately after being deposited, decreased motility by 12 and 24 hours, and improved motility at 36 and 48 hours. In the beginning, spermatozoa motility in the uterus was like ejaculation. The characteristics of motility were different according to time. At 12 hours, spermatozoa showed oscillatory motility with only tail beating and were not progressive. The most striking change of motility was at 36- and 48 hours, when spermatozoa motility was developed, with its head trying to perforate something and its tail beating circularly Fig. 3, as determined by Ccanahuire (2019). Alpaca spermatozoa did not reach hyperactivation but instead activation. Although spermatozoa presented progressive

motility and up to 48 hours, they did not get the state of hyperactivation. The main reason for this change in motility might be due to the absence of a thick seminal plasma at the time of ejaculation and the presence of proteins like glycosaminoglycans, specifically, keratan sulfate, which might be so attached to spermatozoa limiting its movement (Kershaw et al., 2012).

The type of spermatozoa movement was briefly described earlier (Pacheco et al., 2011). In this study, a systematic approach was used, and spermatozoa from slow tail movement changed to head and tail direction by 36 and 48 hours after ejaculation. This change of motility was better expressed in the oviducts. In other species, this kind of motility is called hyperactivation (Overstreet and Cooper, 1979; Suarez et al., 1983). Thus, alpaca spermatozoa are also activated after 36 hours.

The second feature of spermatozoa migration was that their placement in the oviducts appeared to be rapid, as evidenced in rabbits (Overstreet and Cooper, 1978), sheep (Mattner and Braden, 1963), and cows (VanDemark and Moeller, 1951). Alpaca spermatozoa were found in the oviducts at time zero because of the short distance between the uterine horns to the oviduct, 3 to 4 cm. The evaluation time was at most 15 minutes, and consequently, few spermatozoa were already present in the oviduct. Although there could be differences between sections of the oviduct, this was not considered in the present study. In addition, some alpaca spermatozoa are free, which is a second reason for finding spermatozoa within the oviducts. This is comparable to the rabbit (Suarez et al., 1983). And in species of spontaneous ovulation, sheep (Mattner and Braden, 1965), cow (Dobrowolski and Hafez, 1970)

Spermatic concentration within the uterus and oviducts differed between the two reproductive organs. More spermatozoa

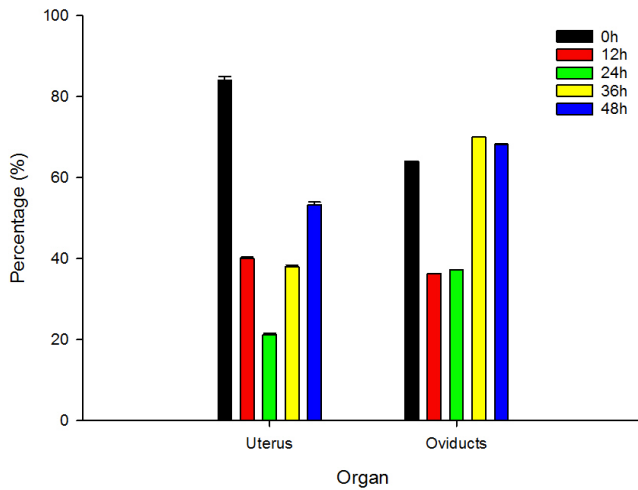


Figure 1. Motility of alpaca spermatozoa at different times, 0, 12-, 24-, 36-, and 48 hours following copulation in the uterus and oviducts of the female.

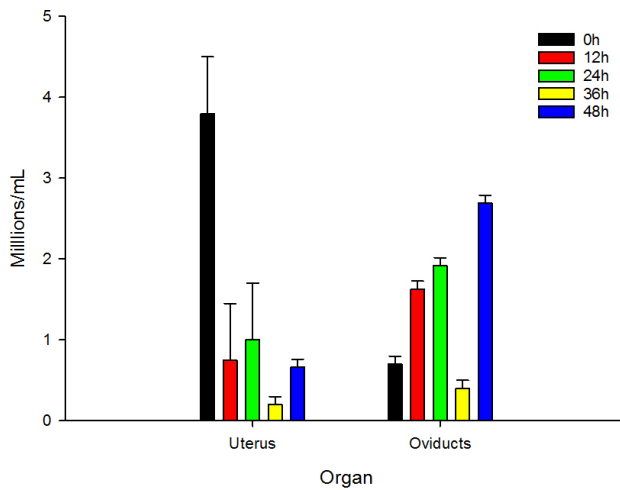


Figure 2. Sperm concentration in the uterus and oviducts of female alpacas following copulation.

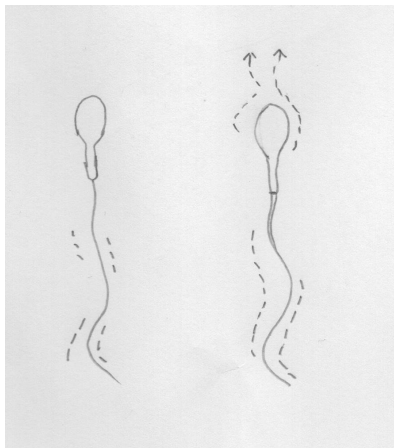


Figure 3. Schematic representation of individual alpaca spermatozoon motility at 0 (time of copulation) and 36 hours from copulation within the female's reproductive tract.

Time (hours)	Uterus	Oviducts
0	Nonprogressive Slow tail beating Many spz adhered to seminal plasma Many spz with no movement	Few spz present Rapid tail beating Some spz with no movement
12 and 24	Slow tail movement Many spz with no movement	Head movement Tail rotatory movement Slow movement
36 and 48	Whole spermatozoa with slow movement	Spermatozoa activated Progressive movement Head and tail movement Head lateral and forward Tail rotatory movement Free spz, not seminal plasma

Table 1. Features of alpaca spermatozoa (spz) motility by reproductive organ and from 0 through 48 hours after copulation.

Characteristics	0 hours	12 hours	24 hours	36 hours	48 hours
Viability, %					
Uterus	99.4	95	96.3	80	95.2
Oviduct	99.8	98	96	98.6	98.3
Normal spz, %					
Uterus	94.4	98.5	96.1	99	97
Oviducts	95.6	99.4	98.9	99.1	99.3
Abnormal heads, %					
Uterus	1.4	0.3	0.8	0.2	1
Oviducts	0.3	0.1	0.3	0.2	0.6
Cytoplasmic droplet, %					
Uterus	0.6	0.2	0.1	0.1	0.1
Oviducts	0.2	0.1	0.1	0.2	0.1
Abnormal tails, %					
Uterus	3.6	1.3	3	0.8	2
Oviducts	4	0.7	0.9	0.5	0.5

Table 2. Alpaca sperm viability and morphology in the uterus and oviducts from 0 through 48 hours after copulation.

were collected from the oviducts within 12 through 48 hours than from the uterus. Initial sperm concentration at time zero was higher in the uterus than in the oviducts. This is natural due to semen deposition in the uterus during copulation. The striking feature is that spermatozoa were two times more concentrated in the oviducts than in the uterus, especially starting 12 hours after copulation. Although there was a significant decrease by 36 hours, concentration was still twice higher in the oviducts than in the uterus. This tendency of more spermatozoa within the oviducts agrees with previous work in the alpaca (Bravo et al., 1996). And with spermatozoa sequestered within the oviductal crypts (Apichela, 2014) and ewes for 24 hours (Hawk et al., 1978). Alpaca spermatozoa concentration was also far below the concentration at ejaculation and time zero; this might be due to loss of spermatozoa in the uterus by other factors like being expelled through the vagina to the outside as demonstrated in the mare (Jones, 1995), phagocytosis and polymorph nuclear cells migration into the uterus lumen and backflow to the vagina (Sinnemaa, 2005), cows (Larsson and Larsson, 1986), pigs (Garcia-Vasquez et al., 2015), horse (Troedsson et al., 1998).

Spermatozoa viability and morphology were similar in the uterus and oviducts throughout the study period. Another feature detected was that a minimal number of abnormal spermatozoa was determined in the oviducts. This could be due to the selecting

process, which might present at the utero tubal junction, as reported in the llama (Apichela, 2009; Apichela et al., 2010), pig (Lovell and Getty, 1968; Flechon and Hunter, 1981), and the horse (Troedsson et al., 1998).

CONCLUSIONS

Alpaca spermatozoa did change in motility and concentration; they did not change in viability and morphology. Alpaca spermatozoa were activated 36 and 48 hours in the oviducts after copulation.

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AUTHOR CONTRIBUTION STATEMENT

MC performed data collection and sample analysis

CZ supervised data collection and analyzed data.

WB conceived the study and wrote the paper.

DECLARATION OF INTEREST

No conflict of interest could be perceived as prejudicing the impartiality of the current manuscript.

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