Small Ruminant Research 133 (2015) 140–147

Contents lists available at ScienceDirect

Small Ruminant Research

journal homepage: www.elsevier.com/locate/smallrumres

Llama (*Lama glama*) semen collection via thermo-electric artificial vagina: Effect of seasonality and collection interval on ejaculate characteristics

Luis B. Ferré[a,*], Genoveva Malik[a], Juan F. Aller[a], Ricardo H. Alberio[a], Cristóbal Fresno[b], Michael E. Kjelland[c]

[a] *Biotechnology of Reproduction Group, National Institute of Agricultural Technology (INTA), Balcarce (7620) Buenos Aires, Argentina*
[b] *Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET)/Facultad de Ingeniería, Universidad Católica de Córdoba, Córdoba (X5016DHK), Argentina*
[c] *Conservation, Genetics & Biotech, LLC, Vicksburg, MS 39182, USA*

ARTICLE INFO

Article history:
Received 11 May 2015
Received in revised form 16 August 2015
Accepted 26 August 2015
Available online 9 September 2015

Keywords:
Ejaculation frequency
Semen collection
Sperm evaluation
Season
Llama

ABSTRACT

Adult male Llamas (*Lama glama*) ($n=6$) were collected using a receptive female and a thermo-electric artificial vagina assembled with a polyethylene collection bag with the following objectives: (a) to develop a reliable and repeatable semen collection technique for Llama, (b) validate semen evaluation tests for Llama, and (c) to determine the effect of collection frequency and season on Llama semen characteristics. First, the semen and sperm variables were recorded and validated through their own repeatability. Semen collection intervals tested were: 1X/week for three weeks and 3X/week for another three weeks, the second collection period occurring after two weeks of sexual rest. The collection frequency of 3X/week significantly reduced ($P<0.05$) the sperm viability, motility, concentration, Total Sperm/Ejaculate, Total Motile Sperm/Ejaculate and Total Live Sperm/Ejaculate, but improved Total Sperm/Week and Total Live Sperm/Week. All recorded variables were significantly higher during the summer in comparison to the spring with the exception of morphology abnormalities, volume, and viability. Also, the 1X/week versus the 3X/week semen collection frequencies did not produce a significant difference in the percent of total motile sperm/week. Based on semen and sperm characteristics evaluated herein, Llama semen collected in the summer was better ($P<0.05$), with regard to the majority of the variables analyzed, than semen collected in the spring.

1. Introduction

South American (SA) camelids (i.e., Llama, Alpaca, Vicuña, and Guanaco), or new world camelids, show unique reproductive characteristics such as induced ovulation, extended copulation, continuous intracornual ejaculation, and extreme viscosity of semen (Johnson, 1989; Bravo and Johnson, 1994; Pugh and Montes, 1994; Vaughan and Tibary, 2006; Adams et al., 2009). Reproductive biology knowledge of these animals is still poorly understood in many aspects.

In recent years, there has been a growing effort to study the male and female behavior characteristics and physiology due to the companion animal market and the production of high quality fiber, i.e., fleece. The lack of genetic and breeding improvement programs, donor performance, male progeny testing and nucleus herds with selected traits for being propagated in domestic camelids has caused suboptimal reproduction efficiency and poor breeding performance compared with other conventional domestic species (Sumar, 1996; Bravo et al., 1997b; Brown, 2000; Urquieta et al., 2005; Adams et al., 2009). The lack of a reliable and repeatable semen collection technique for SA camelids has hampered basic studies on seminal characteristics and therefore, the development of routine breeding technique (Fernández-Baca, 1993; Pugh and Montes, 1994; Del Campo et al., 1995; Lichtenwalner et al., 1996b; Bravo et al., 1997b). Major factors that contribute to this situation

* Corresponding author. Present address: Bovine Embryo Research Group, National Institute of Agricultural Technology (INTA), Ruta Nacional 34, Km 227, Rafaela, Santa Fe (2300), Argentina.
E-mail addresses: ferre.luis@inta.gob.ar (L.B. Ferré), genomalik@gmail.com (G. Malik), alleratucha.juan@inta.gob.ar (J.F. Aller), alberio.ricardo@inta.gob.ar (R.H. Alberio), cristobalfresno@gmail.com (C. Fresno), mkjelland@hotmail.com (M.E. Kjelland).

http://dx.doi.org/10.1016/j.smallrumres.2015.08.016
0921-4488/

are the posture adopted by the SA camelids during mating and the duration of copulation (10–50 min).

Several methods have been used for SA camelids semen collection: intravaginal sheaths, urethral fistulae, electroejaculation and artificial vagina (AV) (San-Martin et al., 1968; Sumar, 1985; Fernández-Baca, 1993; Lichtenwalner et al., 1996b; Bravo et al., 1997b; Giuliano et al., 2008). At present, the AV methodology consisting of an AV mounted inside a dummy female has been adopted as a routine procedure to collect ejaculates with varying degrees of success (Garnica et al., 1993; Garnica et al., 1995; Lichtenwalner et al., 1996a, 1996b; Bravo et al., 1997b). The other aforementioned methods have produced large variations with regard to ejaculate volumes (0.4–6.6 mL), semen concentrations (1000–255,000 sperm/mL), and frequent contamination of the semen sample with urine (Sumar, 1985; Fernández-Baca, 1993; Brown, 2000).

Understanding the reasons for the ejaculate quality variations is important for improving Llama artificial reproductive techniques. Some of the variation in the results from previous studies likely stems from the fact that SA camelids are considered non-seasonal breeders outside of their natural habitat, but in their natural habitat, parturitions are restricted to the rainy months (Bravo et al., 1997a; Flores et al., 2002; Urquieta et al., 2005; Giuliano et al., 2008). The objectives of this study were the following: (a) to develop a reliable and repeatable semen collection technique for Llama, (b) to validate semen evaluation tests for Llama, and (c) to determine the effect of collection frequency and season on Llama seminal characteristics.

2. Material and methods

2.1. Chemicals

All chemicals used in this study were purchased from Sigma–Aldrich Chemical Co., (St. Louis, MO, USA), unless otherwise stated.

2.2. Animals and experimental condition

Adult male Llamas ($n = 6$) 3–6 years of age and weighing 149.67 ± 17.76 kg (mean ± SD) were used in the present study. The males were kept together in a paddock, separate from females, and were fed with *Festuca arundinacea, Lolium perenne, Lolium multifolium* and *Bromus unioloides* pastures and water *ad libutum*. Non-pregnant adult females ($n = 7$) were housed together in a paddock with the same nutritional conditions. Balcarce Experimental Station is located in the southeast of Buenos Aires Province (37°45′S, 58°18′W,130 m above sea level). Climate classification is mesothermic and sub-humid, with an annual precipitation of 650–900 mm.

2.3. Semen collection methodology

All animals used in this research were treated in accordance with the Federation of Animal Science Societies (FASS) guide for use of farm animals in research and teaching (FASS, 2010). Ejaculates were collected using both a thermo-electric artificial vagina (teAV) and a receptive female. The teAV consists of a metal tube 17 cm long × 4.4 cm diameter, a temperature sensor allocated on the wall of the tube, a thermostat, an electric resistance surrounding the tube and a battery source. Females were judged to be receptive when they became ventrally recumbent after exposure to a male. Males were trained to copulate with a receptive female and teAV technician operator at their side. Once the male and the female Llamas took the copulatory position, the teAV operator guided the male's foreskin toward the teAV entrance. The inner artificial vagina pressure was low enough to allow for the entrance of a finger and the temperature remained at 37–38 °C. The beginning and end of collection time was recorded, the beginning was determined when the tip of the penis passed through the total length of the teAV. The teAV maximum collection time was 20 min, which represents the average copulation duration of Llama (Bravo and Johnson, 1994; Bravo and Johnson, 1994a, 1997b). During the entire collection time, the teAV included a polyethylene collection bag [PCB] (Flores et al., 2002). The PCB was protected during collection from cold shock and light (Fig. 1).

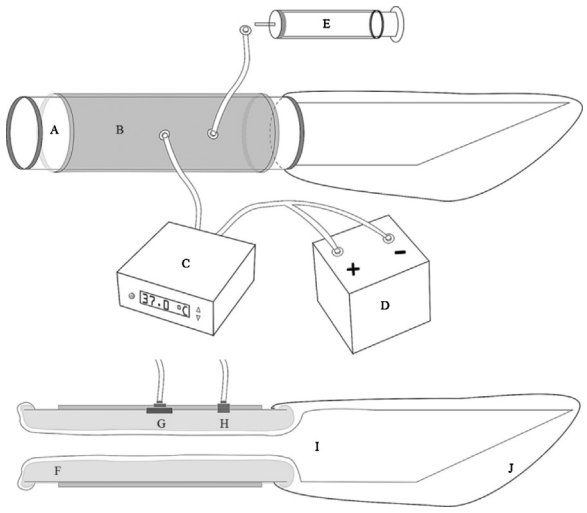

Fig. 1. teAV diagram. (A) artificial vagina body, (B) heating system, (C) temperature controller, (D) 12 V battery, (E) water filling, (F) inner liner cone, (G) temperature sensor, (H) water filling port, (I) polyethylene collection bag and (J) protective/warming cover.

2.4. Season and semen collection frequencies

Two frequencies were compared: one (1X) vs. three (3X) collection times a week. Each frequency was performed during a three week period. Male Llamas were given two weeks of rest between the three week collection periods. Males were assigned randomly to the collection frequency. Testing periods were in spring (October–November) and summer (February–March). Mean temperature, relative humidity and precipitation during October, November, February, and March were: 13.4 °C, 16.8 °C, 19.2 °C, and 19.7 °C, 76.4%, 46.7%, 79.5%, and 78.6%, 94.1 mm, 171.5 mm, 83.5 mm, and 49.8 mm, respectively.

2.5. Semen evaluation

Ejaculate evaluation began approximately 10 min after the collection ended. Notably, the freshly collected ejaculate was always foamy within the polyethylene bag. The ejaculate was placed into a graduated glass tube in a warmed water bath (37 °C) and 10–15 min later, foam became liquid and began to settle to the bottom of the tube.

2.5.1. Subjective assessment of motility

An aliquot of semen was placed on a pre-warmed 25.4 × 76.2 mm glass microscope slide (Andwin, Addison, IL) and overlaid with a 22 × 22 mm #1.5 coverslip (Thomas Scientific, Swedesboro, NJ, USA) and allowed to settle, i.e., stop dispersing across the area under the coverslip. The percentage of motile sperm was determined via subjective evaluation using light microscopy (Nikon Eclipse E100) at 100× magnification, on a heated stage at 37 °C, and analyzing at least five fields of view (Hafez and Hafez, 2000).