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# Comparison of differents methods of sperm selection of llama raw semen

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ABSTRACT

The objective of this study was to compare the efficiency of different sperm selection methods applied to the same llama ejaculate. Four treatments were compared: two variants of the swim up technique (with and without seminal plasma), and two different colloids, Androcoll-E-Large and Percoll®. Using electroejaculation, 21 semen samples were obtained from 7 llama males (n = 7, r = 3). The ejaculates were incubated in a solution of 0.1% collagenase, to decrease thread formation, and then split into 4 aliquots: one aliquot was layered over a column of Androcoll-E-Large (SLC) and the second over a column of Percoll (45%). The third aliquot was deposited in a tube with culture medium and was incubated at a 45° angle for 30 min at 37 °C (SU1). The last aliquot was centrifuged to separate the spermatozoa and seminal plasma. The sperm pellet obtained was resuspended, and transferred to a tube with culture medium which was incubated at an angle of 45° for 30 min at 37 °C (SU2). Both aliquots SLC and P showed higher proportions of progressive motility and plasma membrane functionality ($p \leq 0.05$) than raw semen. There were no significant differences ($p > 0.05$) in sperm viability and in normal spermatozoa between raw semen and treatments. Nevertheless, only SLC did not have a significant increase of bent tails. In conclusion SLC centrifugation would be the method of choice for selecting llama spermatozoa.



## 1. Introduction

Selection of potentially fertile spermatozoa is an event that occurs during sperm migration through the female reproductive tract. The application of assisted reproduction techniques (ART) by-pass, some of these *in vivo* sperm selection mechanisms (intrauterine insemination, *in vitro* embryo production) or may even increase the percentage of non-fertile spermatozoa (damage by cryopreservation),

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thus decreasing pregnancy rates and healthy offspring. Therefore the use of *in vitro* sperm selection techniques is needed to obtain a high percentage of motile spermatozoa, with normal morphology, free from cell debris and dead spermatozoa. In addition, the sperm separation technique should isolate as many motile spermatozoa as possible and should not cause sperm damage or physiological alteration (Henkel and Schill, 2003).

Swim-up and density centrifugation techniques are the most common methods used to select sperm in different species, including humans. Swim-up requires progressive motility of the sperm, because the selection is based on their ability to move from the semen into fresh medium. With this technique, it is possible to obtain a high percentage of motile and morphologically normal sperm. It is a low-cost method, with easy preparation and there is no need to expose spermatozoa to substances that could be toxic. The disadvantage is that the ejaculates should have a high sperm concentration and good motility.

The method of colloid centrifugation relies on highly motile spermatozoa that can penetrate the different layers and pass to the bottom of the tube. Its main advantage is that it can be used with ejaculates of low sperm concentration. Disadvantages are the high cost and the difficulty in preparing density gradients.

As the semen of South American camelids (SACs) characteristically has a high viscosity (Casaretto et al., 2012) is not possible to separate sperm from seminal plasma without performing centrifugation at high speeds (Giuliano et al., 2010). In addition, the sperm of these species do not have progressive oscillatory motility in the native ejaculates (Giuliano et al., 2010). Due to these unique characteristics of the semen, most studies on *in vitro* embryo production and sperm cryopreservation have been done with epididymal spermatozoa (Trasorras et al., 2013; Carretero et al., 2014). Therefore, the development of protocols to prepare spermatozoa from fresh ejaculates is needed. Currently there are no reports about comparative experiments of different sperm selection methods in camelids using the same ejaculate and under the same experimental conditions. The objective of this study was to compare the efficiency of different sperm selection methods applied to the same llama ejaculate. Two variants of the swim up technique (with and without seminal plasma) were evaluated, and a comparison between two different colloids Androcoll-E-Large and Percoll® was done.

## 2. Materials and methods

### 2.1. Animals and location

The study was carried out at the Faculty of Veterinary Sciences of the University of Buenos Aires, in Buenos Aires, Argentina. The city is situated at sea level, latitude 34° 36′ and longitude 58° 26′.

For the study, 7 male *Lama glama* ranging between 6 and 10 years of age and weighing 154.67 ± 19.20 kg (mean ± SD) were used. Animals were kept at pasture in pens and supplemented with alfalfa; they also had free access to fresh water throughout the study. All males were shorn during the month of November.

### 2.2. Semen collection

A total of 21 ejaculates (7 males, 3 ejaculates per male) was collected, processed and evaluated. Semen collections were carried out using electroejaculation (EE) under general anesthesia according to the technique described by Director et al. (2007). All procedures were approved by the Committee for the Use and Care of Laboratory Animals (CICUAL) of the Faculty of Veterinary Sciences of the University of Buenos Aires (protocol 2010/24).

### 2.3. Semen evaluation

The sperm characteristics studied were: sperm motility, membrane function, viability and sperm morphology. Sperm motility was evaluated on a warm stage (37 °C) using a phase contrast microscope (100×); motility was classified as either oscillatory or progressive (Giuliano et al., 2010).

The HOS test for evaluating membrane function was conducted according to Giuliano et al. (2008). Sperm showing the characteristic swelling of the tail were classified as HOS positive, having a functional plasma membrane according to Jeyendran et al., 1992. Morphology was evaluated using phase contrast microscopy (1000×). The staining techniques, using fluorochromes 6-carboxyfluorescein diacetate (CFDA) and propidium iodide (PI) for evaluating membrane integrity (viability), were conducted according to Giuliano et al. (2008). Spermatozoa that fluoresced green throughout their length were classified as being viable (intact membrane) while sperm nuclei that fluoresced red were classified as non-viable (damaged membrane) (described by Harrison and Vickers, 1990). Morphology was evaluated using phase contrast microscopy (1000×) according to Giuliano et al. (2008). In all cases 200 sperm per sample were evaluated. In addition, semen rheological characteristics were evaluated by determining thread formation with a Pasteur pipette, both in the whole ejaculates and in the supernatant of each treated aliquot according to Giuliano et al. (2008).

### 2.4. Semen treatment

Each ejaculate was diluted 4:1 in 0.1% collagenase in H-TALP-BSA medium (Parrish et al., 1986) and incubated 4 min at 37 °C according Giuliano et al. (2010) with the objective of decreasing thread formation and facilitating manipulation of the samples. Afterwards, the ejaculates were divided into four aliquots (between 750 μl and 1 ml each): two aliquots were used for swim up (SU1 and SU2), and two were centrifuged with two different colloids: Percoll® (P) and Androcoll-E-Large (A).

#### 2.4.1. Swim-up techniques (SU)

*2.4.1.1. SU1.* An aliquot of semen was deposited in a 15 ml centrifuge tube and 2 ml of culture medium (Hepes-HAM with of 0.3% m/v bovine serum albumin: H-HAM-BSA) were