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Effects of repeated electroejaculations on kinematic sperm subpopulations and quality markers of Mexican creole goats

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ABSTRACT

Here we show the effects of repeated electroejaculation (EE) on mean values of motility, mitochondrial functionality, and expression of active caspases on goat sperm obtained by EE. Evaluations were done using CASA and flow cytometry. A strategy for identification of kinematic sperm subpopulations, when individual data of sperm are not provided by the CASA system, is provided. Fifty semen samples, five of each of ten adult creole goats, were obtained by electroejaculation. Mean values of total motility, progressive motility and flow cytometry evaluations were compared among EEs. Relationships among mean values of variables were investigated using Spearman correlation and principal component analysis (PCA). For identification of kinematic sperm subpopulations, PCA followed by hierarchical clustering was applied on data of the intervals provided automatically by the CASA system. Total motility does no change after repeated EE. Mean values of motility parameters and molecular markers were unrelated in multivariate space, but bivariate correlations were found. Values in upper and lower intervals defined clearly the sperm subpopulations, which had motility parameters changing over time. Taken together, our results show that repeated EE does not affect mean values of total motility, that molecular markers are not related with motility parameters, and that it is possible to identify kinematic sperm subpopulations when individual data, of motility parameters, are not provided by the CASA system.

1. Introduction

Electroejaculation (EE) is a technique used to obtain semen from animals when normal ejaculation is not possible. Several reports indicate that the quality of sperm obtained by EE may be lower than or not different than the quality of sperm obtained with artificial vagina (Greyling and Grobbelaar, 1983; Jiménez-Rabadán et al., 2012a; Malejane et al., 2014; Marco-Jiménez et al., 2005). In semen samples obtained by EE a high proportion of low molecular weight protein content is present in seminal plasma

Abbreviations: EE, Electroejaculation; CASA, Computer assisted sperm analysis; PCA, Principal component analysis; Tot. Mot., Total motility; Prog. Mot., Progressive motility; VAP, Velocity average path; VSL, Straight line velocity; VCL, Curvilinear velocity; BCF, Beat cross frequency; ALH, Mean amplitude of the lateral head displacement; LIN, Linearity; STR, Straightness.

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(Ledesma et al., 2014); furthermore, sodium levels and concentrations of some proteins in seminal plasma are also altered (Marco-Jiménez et al., 2008). There is no available data on the effects of repeated EE on goat sperm.

There are relatively few studies involving evaluation of goat sperm motility using CASA. The majority of these studies reported mean values of predetermined parameters of motility based on biological criteria, without subjecting the data to the many types of analysis available in statistical packages (Dorado et al., 2010a). Some studies where a CASA system was used to evaluate sperm motility of goats reported only mean values (Álvarez et al., 2012; Jiménez-Rabadán et al., 2012a,b; Kosdrowski et al., 2007). The use of only mean values to describe sperm parameters derived from CASA systems is not an appropriate approach (Abaigar et al., 1999; Holt et al., 2007; Mortimer, 2000) and is of limited value (Dorado et al., 2010a; Martínez-Pastor et al., 2011). In addition to motility, other cellular properties are indicative of sperm quality; apoptotic markers and mitochondrial functionality are two examples (Jiménez-Rabadán et al., 2012b; Pichardo et al., 2010). Although the role of active caspases in ejaculated sperm is not clear, their presence correlates negatively with motility (Gallardo Bolaños et al., 2014; Marchetti et al., 2004; Pichardo et al., 2010). Sperm in an ejaculate form a heterogeneous population, composed of many subpopulations. One such subpopulation contains sperm with active mitochondria (Sousa et al., 2011).

Here we investigated the effects of repeated electroejaculation on parameters of sperm quality and kinematic subpopulations of goat semen, using motility parameters provided by a commercial CASA system.

2. Material and methods

To minimize stress during the experimental procedures, a preconditioning period of 2 months was allowed for the animals to become used to restraint and handling. All handling procedures were performed in accordance with the Ley Protectora de Animales del Estado de México regarding the protection of animals used in scientific experiments and were approved by Bioethic Committee of Centro de Estudios Universitarios Temascaltepec (Letter date: January 3, 2012). All efforts were made to minimize suffering of the animals.

Ten adult creole goats were maintained under uniform nutritional conditions, including tap water *ad libitum*. Five ejaculates were obtained from each goat, one every 2 weeks. Rationale for this schedule of semen sampling was based approximately on the time required for sperm maturation in the epididymis and duration of stages of spermatids, spermatocytes and spermatogonium in the testis (França et al., 1999).

2.1. Semen collection

Semen samples were obtained on the following dates: 04/12/2012 (D1), 04/26/12 (D2), 05/11/2012 (D3), 05/24/2012 (D4), and 06/07/2012 (D5). An electroejaculator ElectroJac®5 (Ideal Instruments®, Lexington, KY, USA) with rectal probe of 2.5 cm diameter, 15 cm in length, and with three linear electrodes of 10 cm each was used.

Animals received an intramuscular injection of detomidine (270 μg/kg body weight) plus ketamine (1.4 mg/kg body weight) 15 min before semen collection, as recommended (Santiago-Moreno et al., 2011). The rectum was cleaned of feces and the preputial area was shaved and washed with physiological saline solution. The rectal probe was lubricated and gently inserted into rectum and orientated so that the electrodes were positioned ventrally. The device was used in automatic setting, applying cycles of stimuli of 2 s with 2 s rest intervals between stimuli. The initial voltage was 1 V and was increased in each series until a maximum of 5 V (Jiménez-Rabadán et al., 2012a,b). The penis was extended beyond the prepuce, and semen was collected into sterile 15 ml polypropylene tubes of and maintained at 37 °C. The electro-stimulation was stopped at time of ejaculation.

Semen was diluted with Tyrode's salt solution (Sigma, St. Louis, MO, USA) and stabilized during 15 min before analysis of motility. Personnel in charge of management of goats, electroejaculator operator, and CASA operator was ever the same.

2.2. Evaluation of sperm motility

Ejaculates were diluted with Tyrode's (Sigma) and held in 37 °C waterbath for 15–20 min before evaluation of sperm motility.

The CASA system used was an IVOS (Hamilton Thorne Biosciences, Beverly, MA, USA) with 10.7s software (Piramid Technical Consultants, Inc., Lexington, MA, USA), plate warmed to 37 °C, negative phase contrast, 10× objective, and magnification 1.89. Settings of the CASA system were as follow: frames per second (Hz) 60, number of frames 30, minimum cell contrast 15, minimum cell size (pix) 8, cell size (pix) 9, cell intensity 125, threshold straightness 80, medium cell VAP cut-off (μm/s) 25, low VAP cut-off 5.0, low straight line velocity (VSL) cut-off (μm/s) 0.0, minimum static intensity gates 0.75, maximum static intensity gates 1.99, minimum static size gates 1.15, maximum static size gates 10.0, minimum elongation gates 0, and maximum elongation gates 99.

Four microliters of diluted semen were put in a Leja4® slide (Leja, Luzernestraat, Netherlands) of 20 μm depth, prewarmed to 37 °C. At least 200 sperm were evaluated per sample (~3–4 fields), and means for each of the following motility parameters were recorded: total motility (%), progressive motility (%), velocity average path (VAP, μm/s), curvilinear velocity (VCL, μm/s), straight line velocity (VSL, μm/s), beat cross frequency (BCF, Hz), linearity (LIN, VSL/VCL × 100, %), straightness coefficient (STR, VSL/VAP × 100, %), and mean amplitude of the lateral head displacement (ALH, μm). Descriptors of sperm motion were previously described (Becerril et al., 2013; Mortimer, 2000). Management of semen aliquots and CASA system operation was realized by a veterinarian experienced in the handling of semen.

The CASA system provided mean values of the motility parameters and histograms with ten predefined intervals for each of the motility parameters (Supplemental File 1);

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