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Influence of semen collection method on sperm cryoresistance in small ruminants

P. Jiménez-Rabadán[a], A.J. Soler[b,*], M. Ramón[a], O. García-Álvarez[b], A. Maroto-Morales[b], M. Iniesta-Cuerda[b], M.R. Fernández-Santos[b], V. Montoro[b], M.D. Pérez-Guzmán[a], J.J. Garde[b]

[a] Regional Center of Animal Selection and Reproduction (CERSYRA), JCCM, Avenida del Vino, s/n, 13300, Valdepeñas, Spain
[b] SaBio Group, National Wildlife Research Institute (IREC), CSIC-UCLM-JCCM, ETSIA, Campus Universitario s/n, 02071 Albacete, Spain

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ABSTRACT

Semen collection for cryopreservation is a key step for small ruminant conservation programs. While in these species semen is mainly collected via artificial vagina (AV), electroejaculation (EE) provides a viable alternative for untrained males. Herein we investigated the effect of semen collection method on post-thaw sperm quality by comparing two small ruminant species, sheep and goats. Semen from Blanca-Celtibérica bucks and Manchega rams was collected by AV and EE on the same day and cryopreserved using a standard protocol. At thawing, sperm motion parameters were evaluated by CASA, whereas membrane stability (YO-PRO-1), sperm viability (propidium iodide, PI) and mitochondrial activity (Mitotracker Deep Red) were analyzed using flow cytometry. The semen collection method negatively affected post-thaw sperm quality in bucks but not in rams. Thus, in bucks, post-thaw sperm motility was higher for samples collected by AV as compared to those obtained via EE. Similarly, post-thaw sperm parameters evaluated by flow cytometry were worse for buck samples collected by EE than those collected by AV in the same species, or than ram samples regardless of collection method. These results suggest that ovine and caprine spermatozoa have a different response to the cryopreservation process depending upon the semen collection method used. We hypothesize that the EE procedure may lead to changes in the composition of the ejaculate in bucks that would make spermatozoa more susceptible to the cryopreservation process, whereas this procedure would have had no effect on ram spermatozoa. This assumption requires further investigation.

1. Introduction

Artificial insemination (AI) in small ruminants is most commonly performed using fresh semen given the low fertility rates typically achieved with frozen spermatozoa. However, sperm cryopreservation is an important tool for breed improvement or conservation programs in various species, including small ruminants. For this reason, numerous studies have been performed in recent years with the goal of optimizing sperm cryopreservation protocols in these species.

While often overlooked, the first important step of the cryopreservation process is that of semen collection. In small ruminants, either an artificial vagina (AV) or electroejaculation (EE) are commonly used methods of semen collection. While collection with an AV is the preferred

* Corresponding author.
E-mail address: anajosefa.soler@uclm.es (A.J. Soler).

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method (Leboeuf et al., 2000), this technique requires a training period (Wulster-Radcliffe et al., 2001). Therefore, EE is considered an acceptable alternative for safely and repeatedly collecting ejaculates when males have not been trained to the AV (Jiménez-Rabadán et al., 2012, 2013, 2015). However, in some species, post-thaw sperm quality may vary depending upon the collection method. For instance, Marco-Jiménez et al. (2005) reported that ram spermatozoa collected by EE were more resistant to cryodamage than when collected by AV. Conversely, another laboratory reported better post-thaw buck sperm quality for samples collected via AV vs. EE (Jiménez-Rabadán et al., 2012). Collection by EE may change the secretory function of one or more of the accessory sex glands thus modifying the overall composition of the seminal plasma (Marco-Jiménez et al., 2008). Moreover, the protein composition and concentration in seminal plasma may vary depending upon the collection procedure. Notably, some of these proteins may play important roles in preventing cold-shock damage (Barrios et al., 2005) and changes in their relative proportion in seminal plasma may therefore influence the cryoresistance of sperm samples.

The small ruminant denomination includes all sheep and goats as well as some wild species. For this reason, sheep and goats are often lumped together as one species when developing and applying assisted reproductive technologies. For example, most sperm cryopreservation protocols used for caprine have been extrapolated from those developed for the ovine species (Chemineau et al., 1991; Parkinson, 2009; Shipley et al., 2007), and thus are very similar in regards to extenders, cryoprotectants and cooling rates (Salamon and Maxwell, 1995, 2000). Nevertheless, differences may exist between small ruminant species in regards to the response of spermatozoa to the cryopreservation procedure. Furthermore, studies assessing the effect of the semen collection method on post-thaw sperm quality in bucks (Jiménez-Rabadán et al., 2012) and rams (Álvarez et al., 2012; Marco-Jiménez et al., 2005), are not directly comparable given different semen handling techniques and cryopreservation protocols.

Therefore, the objective of the present study was to examine the effect of semen collection method on sperm cryopreservation results in two phylogenetically close-related species of small ruminants when using the same freeze-thaw protocols. The results obtained may help increase our understanding of the changes that occur during the cryopreservation procedure as related to the semen collection method. Moreover, cryopreservation protocols may be modified in a species-dependent manner according to the collection method available in order to optimize post-thaw sperm survival.

2. Materials and methods

2.1. Animals and reagents

Animal handling was performed in accordance to Spanish Animal Protection Regulation, RD 53/2013, which conforms to European Union Regulation 2010/63. Seven males of the Blanca-Celtibérica goat breed (age >1.5 years) and five males of the Manchega sheep breed (age >1.5 years) were used. All males were maintained and managed at the Regional Center of Animal Selection and Reproduction (CERSYRA) located in Valdepeñas (Spain). Sperm thawing and analysis was conducted in the laboratory of the SaBio group in Albacete (Spain).

Chemicals were of reagent grade and purchased from Sigma (Madrid, Spain). Biladyl® was purchased from Minitüb (Tiefenbach, Germany). Fluorescence probes were purchased from Invitrogen (Barcelona, Spain), except for propidium iodide (PI) which was purchased from Sigma. Chromatographically purified acridine orange (AO) was purchased from Polysciences Inc. (Warrington, PA, USA).

2.2. Semen collection

For all 12 males in the study, ejaculates were collected twice on the same day, that is, once with each the AV and EE method, respectively, and following the same protocols, to ensure that results would be comparable between both species and collection methods. Prior to initiation of this study, all males were trained to the use of an AV with semen collections performed routinely once a week. For this study we performed 2 semen collections (replicates) for each collection method. At each experimental day, semen was collected first via AV, followed by EE 2 h later. The EE method was carried out following the protocol previously described by our group (Jiménez-Rabadán et al., 2012, 2015). Males were anesthetized with xylazine (0.2 mg/kg Rompun® 2% i.m.; Bayer S.A., Barcelona, Spain), the rectum was cleaned of feces and the prepucial area was shaved and washed with saline solution. A three electrode probe connected to a power source that allowed for voltage and amperage control was used (P.kT. Electronics, Boring, OR, USA). Probe diameter, probe length and electrode length were 3.2, 35.0 and 6.6 cm, respectively. The EE regime consisted of consecutive series of 5-s pulses of similar voltage, each separated by a 5-s break. Each series consisted of a total of four pulses. The initial voltage was 1 V which was increased in each series until a maximum of 5 V. Urine contamination was tested and ejaculates with urine were discarded.

2.3. Semen evaluation

Immediately after semen collection, the ejaculate volume (measured in a conical graduated tube) and sperm concentration (by spectrophotometer) were evaluated, and total number of spermatozoa (STN) was calculated accordingly (volume × concentration). The percentage of motile spermatozoa (SM) was evaluated subjectively in aliquots of semen diluted (1:200) in a phosphate buffer saline (PBS) and incubated for 5 min at 37 °C, using a phase-contrast microscope (×100).

2.4. Semen cryopreservation

All ejaculates were diluted in a commercial extender, Biladyl® (20% clarified egg yolk), following the two-step dilution method. First, the prewarmed (30 °C) non-glycerolated fraction was added and the diluted sample was cooled to 5 °C for 2 h. Then, samples were further