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Single layer centrifugation (SLC) improves sperm quality of cryopreserved Blanca-Celtibérica buck semen

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ABSTRACT

The aim of the present study was to evaluate the effect of sperm selection by means of single layer centrifugation (SLC) on sperm quality after cryopreservation, either when SLC is used before freezing or after thawing, using Blanca-Celtibérica buck semen collected by electroejaculation (EE). Ejaculates from six bucks were collected by EE and divided into two aliquots. One of them (unselected) was diluted with Biladyl® by the two-step method and frozen over nitrogen vapor. The other aliquot was selected by the SLC technique and subsequently frozen in the same way as the unselected samples (SLC before freezing). In a further treatment, two unselected straws were thawed and SLC was carried out (SLC after thawing). At thawing, sperm motility of all samples ((i) unselected; (ii) selected before freezing and (iii) selected after thawing) was evaluated by CASA. In addition, integrity of the plasma membrane, mitochondrial membrane potential, ROS production and DNA fragmentation index were assessed by flow cytometry. Most of the sperm parameters were improved ($P \leq 0.001$) in samples selected by SLC after thawing in relation to unselected or selected by SLC before freezing. The percentage of progressive motile spermatozoa was greater (86%) for sperm samples selected after thawing compared with unselected (58%) or selected before freezing (54%). Moreover, percentages of spermatozoa with intact plasma membrane and spermatozoa with high mitochondrial membrane potential (hMMP) were also greater for sperm samples selected after thawing compared to sperm samples unselected or selected before freezing (spermatozoa with intact plasma membrane: 80% vs. 32% vs. 12%; spermatozoa with hMMP: 54% vs. 1% vs. 15%; respectively). Therefore, sperm quality after cryopreservation is improved in Blanca-Celtibérica buck ejaculates collected by EE when a sperm selection technique such as SLC is carried out after thawing.

1. Introduction

The Blanca-Celtibérica goat is an autochthonous breed from Spain considered to be endangered. Endangered breeds must be preserved by conservation methods, one of which is the development of genetic resource banks. In genetic resource banks, gametes and embryos are cryopreserved allowing genetic resources to be stored indefinitely (Watson and Holt, 2001).

Semen from domestic males is usually collected by artificial vagina (AV) (Leboeuf et al., 2000), but this technique requires a previous training period (Wulster-Radcliffe et al., 2001). Electroejaculation (EE) is another method of semen

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collection which is an alternative when males are not trained for an AV, or for wild species, and may be a viable method of repeatedly collecting semen from the same individuals without resulting in the death of the animal (Santiago-Moreno et al., 2009).

The Blanca-Celtibérica goat breed is bred and reared in extensive systems. In the case of animals breeding in the field, different strategies are needed instead of routine reproductive techniques. In these cases, EE could be used as an alternative collection method to obtain semen because of the difficulty of training animals to use an AV under field conditions. To date, the majority of studies carried out for cryopreserving buck sperm have used samples collected by AV; few of them have used other semen collection methods in this species. Some authors have reported, in various species, higher volume and lower concentration when ejaculates are collected by EE (Marco-Jiménez et al., 2005, 2008; Memon et al., 1986), which could be due to an increased contribution from the accessory sex glands because of electrical stimuli (Mattner and Voglmayr, 1962). Moreover, the EE procedure changes the secretory function of one or more accessory glands modifying the composition of the seminal plasma, as has been demonstrated in other species (Marco-Jiménez et al., 2008). It is possible that this fact could affect the cryosurvival of sperm samples obtained by EE. The only study in goats which compared the effect of semen collection method (AV vs. EE) on sperm cryopreservation demonstrated lower sperm quality after cryopreservation for samples obtained by EE in relation to those obtained by AV (Jiménez-Rabadán et al., 2012).

In addition, there is a specific problem for buck semen cryopreservation when samples are extended in diluents containing egg yolk. Seminal plasma has been shown to contain a phospholipase secreted by the accessory bulbourethral gland which catalyses the hydrolysis of lipids in egg yolk to fatty acids and lysophospholipids, which are toxic to spermatozoa (Aamdal et al., 1965; Iritani and Nishikawa, 1963; Sias et al., 2005). Many studies have reported that a washing procedure for removing the seminal plasma from goat ejaculates is necessary to increase sperm quality after freezing and thawing (Kozdrowski et al., 2007; Machado and Simplicio, 1995), although Jiménez-Rabadán et al. (2012) showed no improvement on sperm quality after cryopreservation in Blanca-Celtibérica buck when semen samples were washed.

Sperm selection methods have been used in order to remove seminal plasma or extenders from the spermatozoa, and also to enrich the amount of cells with normal morphology and/or motility for their later use in the different reproductive techniques (Hollinshead et al., 2004; Mortom et al., 2006). Recently, a sperm selection technique using single layer centrifugation (SLC) has been reported, with the ability to select effectively good quality spermatozoa with a shorter preparation time and less complicated process than the conventional density gradient centrifugation (DGC) (Morrell and Rodríguez-Martínez, 2009). In this SLC technique, spermatozoa are centrifuged through a column (single layer) of glycidoxypropyltrimethoxyl silane-coated silica in a species-specific formulation (Androcoll^{TM}), resulting in the selection of motile, morphologically normal spermatozoa with intact membranes and good chromatin integrity (Morrell et al., 2009a, 2009b). SLC selected a sperm sub-population with high quality and fertility from frozen–thawed stallion and bull semen (Macías-García et al., 2009; Thys et al., 2009). Since semen collected by EE is considered to have a higher amount of seminal plasma and with a different composition compared to semen collected by an AV (Marco-Jiménez et al., 2005, 2008), and if seminal plasma from buck males is detrimental in egg-yolk based freezing extender, the use of SLC to separate the spermatozoa from seminal plasma, in semen collected by EE could improve the sperm parameters after cryopreservation.

With this background, the effect of sperm selection by means SLC through Androcoll-B (a colloid for bull spermatozoa) on goat sperm quality after cryopreservation was studied, either when the SLC was used before freezing or after thawing for semen samples collected by EE.

2. Materials and methods

2.1. Animals

All animal procedures were performed in accordance with Spanish Animal Protection Regulations, RD 1201/2005, which conforms to European Union Regulation 2010/63. Six males of Blanca-Celtibérica goat breed (age > 1.5 years) were used. Males were maintained and managed at the Regional Center of Animal Selection and Reproduction located in Valdepeñas (Spain).

2.2. Semen collection

Ejaculates from 6 males were collected by EE. The EE procedure was carried out using the protocol described by Garde et al. (2003). Males were anaesthetized with xylacine (0.2 mg/kg Rompun® 2% i.m.; Bayer S.A., Barcelona, Spain), the rectum was cleaned of faeces, and the preputial area was shaved and washed with physiologic saline serum. A three electrode probe connected to a power source that allowed voltage and amperage control was used (P.T. 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 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. Contamination for urine was tested and ejaculates where urine was present were discarded. Ejaculates were collected weekly during the breeding season (September–December) with three ejaculates per buck being obtained for the present study.

2.3. Evaluation of ejaculates

Immediately after semen collection, the volume of the ejaculate (measured in a conical graduated tube), sperm concentration (by spectrophotometer) and wave motion (scored on a scale of 0–5) were evaluated. 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). The percentage of