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Use of Androcoll-S after thawing improves the quality of electroejaculated and epididymal sperm samples from red deer

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ABSTRACT

Single Layer Centrifugation is a useful technique to select sperm with good quality. The use of selection methods such as Androcoll could become an important tool to improve the quality of sperm samples and therefore to improve other artificial reproductive techniques such as sperm sex sorting, in vitro *fertilization* or AI. The aim of this study was to evaluate the effect of a Single Layer Centrifugation with Androcoll-S on the sperm quality of red deer sperm samples of two different origins, electroejaculated samples and epididymal samples obtained post-mortem, after thawing and after an incubation for 2 h at 37 °C. Sperm motility, viability, membrane permeability, mitochondrial activity, acrosomal status and DNA fragmentation were determined for all samples. The samples selected by Androcoll-S showed an improvement in sperm kinematics compared to unselected samples after thawing and after incubation. The same effect was observed in parameters such as viability, mitochondrial activity or acrosomal status which were improved after the selection. In contrast, no difference was found in DNA fragmentation between selected and unselected samples within the same sperm type. We conclude that sperm selection by SLC with Androcoll-S after thawing for red deer sperm of both types is a suitable technique that allows sperm quality in both types of sperm samples to be improved, thereby improving other assisted reproductive techniques. Further studies (IVF and in vivo fertilization) are required to determine whether this improvement can increase fertility, as has been shown for other species.

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1. Introduction

During the last few decades the use of artificial reproductive techniques for wild ungulates has become important not only for conservation purposes (Jabbour et al., 1997; Pukazhenthi and Wildt, 2003) but also for economic purposes (deer meat, velvet and trophies), since some species are commercially important in several countries (Asher et al., 1999). The increasing use of artificial insemination (AI) with frozen-thawed sperm in red deer requires improved sperm quality in frozen samples. Cryopreservation protocols have to be adjusted to take into account the differences between samples obtained by electroejaculation and those obtained post-mortem from

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the epididymal cauda. Previous studies (Garcia-Macias et al., 2006) assessed differences between ejaculated and epididymal samples. Moreover, some studies (Fernandez-Santos et al., 2007; Domínguez-Rebolledo et al., 2010; Anel-López et al., 2012) were carried out to improve sperm quality by the addition of antioxidant in the extender. Since both electroejaculation and epididymal sperm samples are sources of sperm for different artificial reproductive techniques in this species, it is important to assess the effect that Single Layer Centrifugation with Androcoll has on such samples.

The use of selection methods such as Androcoll could become an important tool to improve the quality of sperm samples and therefore to improve other artificial reproductive techniques such as sperm sex sorting, in vitro *fertilization* (IVF) or AI. During the sex sorting process, the slow speed of sorting is a significant problem due to samples with high numbers of dead sperm. The correct application of Androcoll centrifugation before the sex sorting process could improve sorting speed due to an increase in the proportion of viable sperm in some samples. In addition, the use of selection methods enables removal of all the freezing extender from the sample, allowing the use of thawed samples without contamination with cryoextender (Hollinshead et al., 2004; Morton et al., 2006). Thus, it is necessary to check the capability of Androcoll-S to select red deer sperm. Processing with Androcoll is easier than other methods such as discontinuous density gradient centrifugation or swim up. The use of Androcoll® with species-specific colloid formulations has been successfully reported in stallion (Macías García et al., 2009), bull (Thys et al., 2009) or buck (Jiménez-Rabadán et al., 2012).

The aim of this study was to evaluate the effect of Androcoll on sperm quality of thawed red deer samples obtained by two collection methods; electroejaculated samples (EE) and epididymal samples obtained post-mortem (EP).

2. Materials and methods

2.1. Reagents and media

Fluorescence probes YO-PRO-1 and Mitotracker Deep Red were purchased from Invitrogen (Barcelona, Spain), propidium iodide (PI) and PNA-FITC were acquired from Sigma (Madrid, Spain) and acridine orange (chromatographically purified) was purchased from Polysciences (Warrington, PA, USA). Stock solutions of the fluorescence probes were: PI: 7.5 mM; PNA-FITC: 0.2 mg/mL; YO-PRO-1: 50 μM; Mitotracker Deep Red: 1 mM. All fluorescent stocks were prepared in DMSO—except for PI and PNA-FITC, which were prepared in water—and kept in the dark at −20 °C until needed. The stock solution of acridine orange was prepared in water at 1 mg/mL and kept in the dark at 5 °C. Flow cytometry equipment, software and consumables were purchased from Beckman Coulter (Fullerton, CA, USA) or Becton Dickinson (San Jose, CA, USA). The stock solutions of the antioxidants were prepared at 100 mM in DMSO (Trolox) or in water (reduced glutathione, GSH) and stored at −20 °C.

The work medium for cytometry assessment was bovine gamete medium (BGM-3) composed of 87 mM NaCl, 3.1 mM KCl, 2 mM $CaCl_2$, 0.4 mM $MgCl_2$, 0.3 mM NaH_2PO_4, 40 mM HEPES, 21.6 mM sodium lactate, 1 mM sodium pyruvate, 50 μg/mL kanamycin, 10 μg/mL phenol red and 6 mg/mL BSA (pH 7.5). Solutions for SCSA (Sperm Chromatin Structure Assay) were prepared as follows (Evenson and Jost, 2000): TNE buffer (0.01 M Tris–HCl, 0.15 M NaCl, 1 mM EDTA, pH 7.4), acid-detergent solution (0.17% Triton X-100, 0.15 M NaCl, 0.08 N HCl, pH 1.4) and acridine orange solution (0.1 M citric acid, 0.2 M Na_2HPO_4, 1 mM EDTA, 0.15 M NaCl, pH 6.0; acridine orange was added from the stock up to 6 μg/mL). These solutions were kept at 5 °C in the dark.

2.2. Ejaculate collection and cryopreservation

Samples were obtained from nine mature stags during the breeding season (mid-September). Animals were housed in a semi-free ranging environment at Las Lomas Farm (Medianilla S.L., Cadiz, Spain). Animal handling and electroejaculation were performed in accordance with Spanish Harvest Regulation RD 1201/2005 that conforms to European Union regulation 2010/63. The electroejaculation procedure was carried out as previously described (Martínez et al., 2008). Males were anesthetized with Xylacine (0.75 mg/kg) (Rom-pun® 2%; Bayer AG, Leverkusen, Germany). The rectum was cleared of faeces and the preputial area was shaved and washed with physiological saline solution. 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 electroejaculation regimen consisted of consecutive series of 5 pulses of similar voltage and separated by 5 s. The initial voltage was 1 V and was increased in each series up to a maximum of 5 V. Semen was collected in fractions in graduated glass tubes. Sperm concentration was assessed using a hemocytometer (Bürker chamber; Brand GmbH, Germany), after diluting the sample in a glutaraldehyde solution (5 mL of sample in 500 mL of 2% glutaraldehyde solution—29 g/L glucose monohydrate, 10 g/L sodium citrate tribasic dihydrate and 2 g/L sodium bicarbonate). We discarded the fractions with urine contamination that were positive to Urea Test Strips (Diagnostic Systems GmbH, Holzheim, Germany). Fractions with total motility under 80% were discarded.

Seminal samples were extended to a final concentration of 100×10^6 sperm/mL using Triladyl® (Minitüb, Tiefenbach, Germany) supplemented with 20% (v/v) of egg yolk (EY). Then sperm samples were immersed in a programmable temperature-controlled water bath (Programmable Model 9612, PolyScience, Niles, IL, USA) and slowly cooled from 21 °C to 5 °C over 90 min, and left for an equilibration time of 2 h. After this period semen was packaged in 0.25 mL straws (Minitüb, Tiefenbach, Germany) and frozen in nitrogen vapour (4 cm above liquid nitrogen) for 10 min, before plunging into liquid nitrogen for storage.

2.3. Animals, epididymal collection and cryopreservation

For this study, we used spermatozoa recovered from the epididymides of nine mature stags that were legally hunted

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