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Undiluted or extended storage of ram epididymal spermatozoa as alternatives to refrigerating the whole epididymes

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ABSTRACT

The effect of storage procedure at 5 °C on the quality of ram spermatozoa from the cauda epididymis was analyzed. Two strategies were tested at 0, 24, 48 and 72 h post-mortem: (1) spermatozoa held in the epididymal fluid and stored either in the cauda epididymis (In-EPID) or in vitro (Ex-EPID), (2) epididymal spermatozoa extended in three media at 320, 370 and 420 mOsm/kg (D320, D370, D420). Analyzed parameters were: osmolality, pH, motility, acrosomal status and viability. In experiment 1, osmolality of the In-EPID samples, but not in Ex-EPID, increased with post-mortem time. Motility of In-EPID spermatozoa in samples, after 24 h post-mortem, was higher compared to the Ex-EPID samples, although differences decreased at 48 and 72 h. In experiment 2, total (TM) and progressive motility (PM) were not significantly affected by storage time for D320 and In-EPID samples. However, the motility of D370 and D420 samples significantly decreased with time. TM and PM of D320 were significantly higher than D370 and D420 at 72 h. At 24 h, sperm viability was higher for In-EPID (80.7 ± 3.4%) than for the extended samples (44.8 ± 2.9%, 37.7 ± 3.9% and 48.6 ± 6.0% for D320, D370 and D420, respectively), which also decreased faster with time. At 24 h, the percentage of damaged acrosomes was low and similar for the four methods of storage, but damaged acrosomes increased with time for D320 and D370. Storing the spermatozoa in the epididymis is a good strategy for maintaining sperm quality in ram, at least for 48 h. The D320 extender preserve motility of epididymal spermatozoa but does not protect the status of the acrosome.

1. Introduction

In the last decade the conservation of rare breeds of domesticated species has been very difficult and many of them have disappeared or are going to become extinct (Canali, 2006). This loss of biodiversity has been due to a poor management policy of genetic resources. In Spain, there is a wide range of animal genetic resources due to its specific geographical and climatic characteristics. These resources are threatened by the introduction of alien breeds with high production rates, and as an example we can cite the 34 sheep breeds officially declared endangered. This situation has motivated the implementation of a National Programme for conservation, improvement and promotion of livestock breeds (Royal Decree 2129/2008). This programme recommended establishing a germplasm bank for certain sheep breeds with increased risk of losses of genetic variability. This bank would ideally contain

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germplasm systematically collected from living animals. However, genetically valuable animals may die unexpectedly, calling for a methodology to collect their germplasm post-mortem. In that event, the post-mortem collection of epididymal spermatozoa allows the conservation of valuable genetic material that would otherwise be lost (Saragusty et al., 2006). Epididymal spermatozoa provide a sufficient quantity of viable spermatozoa to be used to fertilize oocytes with the resulting zygotes being able to develop into live young (Songsasen et al., 1998; Sankai et al., 2001; Soler et al., 2003a; Martins et al., 2009).

However, sperm in the epididymis are viable only for a certain period of time and then degenerate rapidly (Hishinuma et al., 2003; Kaabi et al., 2003; Soler et al., 2003b; Yu and Leibo, 2002). Previous studies in several species have indicated that storage of epididymides at 5 °C may be an appropriate way to maintain sperm motility and fertilizing capacity for several days (mice: An et al., 1999; cat: Ganan et al., 2009; boar: Kikuchi et al., 1998; mouse: Kishikawa et al., 1999; red deer: Martinez-Pastor et al., 2005a; bull: Martins et al., 2009; dog: Yu and Leibo, 2002).

Kaabi et al. (2003) preserved ram epididymides at 5 °C, finding good sperm viability until 48 h post-mortem, although their in vitro fertility potential declined significantly after 24 h. Similarly, Martinez-Pastor et al. (2005b) found that deer sperm quality obtained from the epididymis was mostly maintained for the first two days.

In studies examining the conservation of epididymal sperm, the epididymides are usually transported to the laboratory from the site of slaughter at 5 °C. In this period, alterations in the physical environment of the epididymal sperm may cause a loss in sperm quality. Histological examination of mouse epididymides revealed that distinct degenerative changes did not occur until 12 h postmortem (Songsasen et al., 1998) when the epithelial cells became pyknotic and released their intracellular contents into the lumen of the epididymides. By 24 h postmortem, the structure of the epididymal tubule appeared to be breaking down. Martinez-Pastor et al. (2005b) found that osmolality of the epididymal media in red deer increased with postmortem time.

If the epididymis changes its structure after 12 h postmortem, it is possible that the epididymal microenvironment then begins to be harmful to sperm. We hypothesized that extracting the spermatozoa in the field, refrigerating them either undiluted or extended, and keeping them so for several days post-mortem might represent an advantage, comparing to the traditional methodology of storing the whole epididymes. Therefore, these could be alternative strategies for maintaining the quality of post-mortem samples, when immediate cryopreservation is not possible (Sankai et al., 2001; Fernandez-Santos et al., 2009).

The objective of this study was to analyse the effect of storage at 5 °C on the quality of ram epididymal sperm by: (1) evaluation of spermatozoa held in the epididymal fluid and stored either in the organ or in vitro, (2) analysis of epididymal spermatozoa extended in the same medium at different osmolalities.

2. Materials and methods

2.1. Reagents

All the products were obtained from Sigma (Madrid, Spain), except for the SYBR-14 (LIVE/DEAD Sperm Viability Kit) and YO-PRO-1 fluorescence probes, which were acquired from Invitrogen (Barcelona, Spain).

2.2. Animal and sample collection

Testes were collected from fifteen rams (Churra breed) after slaughter, and transported in a refrigerated cooler (5 °C) to the laboratory (University of León) within the first 2–4 h post-mortem. In a cold room, the caudae epididymides were isolated, the superficial vessels were cut to reduce blood contamination and the organ was kept at 5 °C.

2.2.1. Experimental design

Two experiments were carried out. In experiment 1, we compared sperm quality, osmolality and pH among intra-epididymal and undiluted extra-epididymal storage (9 + 9 epididymides). In experiment 2, we tested the effect of dilution of the epididymal spermatozoa in three extenders of increasing osmolality (320, 370 and 420 mOsm/kg), using intra-epididymal stored samples as the control group (6 epididymes for control and 6 for dilution in the three extenders). These three osmolalities were chosen according to previous experiment and represent a hypo-osmotic, iso-osmotic and hyper-osmotic medium, respectively, comparing to the physiological osmolality of the caudal epididymal fluid (experiment 1: the mean value is 365 (isotonic value), with a range of distribution of 355 (25th percentile) to 381 (75th percentile)).

2.2.2. Sample collection

In each cauda epididymes, two symmetrical parts were isolated by a clamp. Experiment 1: one part was maintained without manipulation and the sperm mass was obtained at the different sampling times (0, 24, 48 and 72 h) performing sequential cuts in defined areas of the surface with a scalpel (Intra epididymal sample, In-EPID). The other portion was processed by cuts to obtain the total sperm mass in a glass tube where it was kept undiluted (Extra epididymal sample, Ex-EPID) and samples were obtained sequentially in different time periods (24, 48 and 72 h).

Experiment 2: one part of each cauda was kept as the control (In-EPID) and was manipulated as described in the previous experiment. In the other portion, the total sperm mass was obtained by cuts and this sample was aliquoted and diluted with the same volume of each TTF media (TES-Tris-fructose, pH 7.2, 20% egg yolk and 8% glycerol) with different osmolalities (320, 370 and 420 mOsm/kg, obtained by varying fructose). These diluted aliquots were coded as D320, D370, D420, respectively. Each aliquot was sampled sequentially for analysis each 24 h (24, 48 and 72 h). TTF media containing glycerol, since the epididymal spermatozoa were prepared for freezing.

Between sampling times, the portions of epididymides used for the control samples (In-EPID) were wrapped with