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Short communication

Cryopreservation of epididymal bovine spermatozoa from dead animals and its uses *in vitro* embryo production

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Abstract

The present study aimed to evaluate viability and *in vitro* fertilizing ability of cryopreserved epididymal spermatozoa obtained from dead animals. To collect spermatozoa, epididymides from three males (Bulls A1, A2 and A3) were collected at a local slaughterhouse. As a reference ejaculate from a bull with known in vitro fertility, was used. Sperm characteristics (motility, chromatin and acrosome integrity) were evaluated before and after cryopreservation. Then, frozen spermatozoa from all animals were used for *in vitro* fertilization. Cleavage and blastocyst rates at 48 h (day 2) and 168 h (day 7) post *in vitro* insemination, for bull A1 (82.1 and 38.6%) and A2 (80.7 and 33.8%) were similar ($P > 0.05$) to the reference bull (88.9 and 57.2%). Bull A3 had the lesser cleavage (42.0%) and blastocyst (26.1%) rates. The results showed that epididymal spermatozoa from dead animals can be successfully cryopreserved and used *in vitro* production of embryos.

Keywords: Bovine; Cryopreservation; Epididymal sperm; Genes

1. Introduction

Genetic material either from animals of economical interest or from wildlife can be lost anytime by unexpected death of the animal. In this case, efforts can occur to avoid the total lost of that genetic material, which can be achieved by using reproductive assisted techniques.

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One possibility to preserve the germplasm from dead males is the recovery of spermatozoa from the epididymis. This *post-mortem* procedure has been considered as a very important tool for enhanced utilization of germplasm from valuable animals or animals in risk of extinction (Kaabi et al., 2003).

Although the use of fresh spermatozoa recovered from the epididymis is an alternative procedure, cryopreservation of spermatozoa allows a more efficient and economical way to use that material, because it can be utilized anytime not just after death of an animal.

Based in the importance of that procedure for bovine production and conservation, the present study was designed to evaluate the viability and fertilizing ability of cryopreserved epididymal spermatozoa obtained from dead animals.

2. Materials and methods

2.1. Epididymides collection and transportation

Testis of crossbreed bulls were collected at the abattoir 2 h after the death of the animal, and then epididymides were separated from the testis. Isolated epididymides were placed in 50 mL tubes containing Tyrode's albumin lactate pyruvate medium (TALP) (Parrish et al., 1995) and were transported to the laboratory at room temperature. The time between the removal of the epididymis and arrival at laboratory was 5 h.

2.2. Recovery and cryopreservation of spermatozoa from the epididymis

In the laboratory, the epididymides were washed with alcohol 70%. Various incisions in the tail of epididymis were performed and then, by pressing that region manually the spermatozoa were released and collected. The recovered spermatozoa were placed in a 15 mL tube and diluted with egg yolk Tris glycerol extender as described by Cormier et al. (1997). Then, they were loaded into 0.25 mL straws in a total concentration of 20×10^6 cells and equilibrated for 4 h at 5 °C. After the equilibration period the straws were cooled down to a temperature between −80 and −120 °C, in which they were kept for 20 min. Finally the straws were immersed in liquid nitrogen and stored until their use.

2.3. Sperm evaluation

All sperm samples were evaluated for motility, concentration, morphology, acrosome and chromatin integrity. The percentage of motile spermatozoa was assessed subjectively on a warmed glass-slide immediately after sperm recovery and dilution with cryopreservation medium. Concentration was determined in a hemocytometer in a 1:200 dilution and results are presented as sperm cell/mL. Sperm morphology was evaluated using a phase contrast microscope in 1000× magnification according to Barth and Oko (1989). A total of 200 cells were counted and the results are expressed in percentage.

For acrosome integrity evaluation, spermatozoa were washed twice in TALP medium through centrifugation at 700 × *g* for 6 min. The *pellet* was resuspended with 0.5 mL of TALP and three smears from each sample were made. The smears were fixed in methanol for 5 min, air-dried and stained overnight with Giemsa at 10%. Presence of acrosome was assessed in bright field microscope in 1000× magnification. A total of 200 cells were analyzed and the results are express as percentage.