Comparison of Apoptotic Index Between Cryopreserved Ejaculated and Epididymal Sperm in Stallions

Gabriel Augusto Monteiro, DVM, MSc; Camila de Paula Freitas-Dell’Aqua, DVM, MSc; Priscilla Nascimento Guasti, DVM, MSc; Fernanda da Cruz Landim-Alvarenga, DVM, PhD, MSc; José Antonio Dell’Aqua Junior, DVM, MSc, PhD; Marco Antonio Alvarenga DVM, MSc, PhD*; and Frederico Ozanam Papa DVM, MSc, PhD

The development of a reliable technique to freeze epididymal semen would be a unique alternative to preserve valuable genetic material from unexpectedly lost stallions. Authors’ address: Department of Animal Reproduction and Veterinary Radiology, School of Veterinary Medicine and Animal Science, UNESP, Botucatu, SP 18610-970, Brazil; e-mail: monteiroga@yahoo.com.br. *Presenting author. © 2011 AAEP.

1. Introduction

The aim of this work was to compare the apoptotic index of sperm obtained from three groups—Group 1 (G1): the ejaculated sperm; Group 2 (G2): sperm recently recovered from epididymides; Group 3 (G3): epididymal sperm stored at 5°C for 24 h.

2. Materials and Methods

For G1, two ejaculates from each of seven stallions were collected and then submitted to cryopreservation using BotuCrio™ extender; 1 wk after the last semen collection, stallions were submitted to bilateral orchiectomy, and sperm from one cauda epididymides was harvested immediately after castration (G2). The remaining testicle was stored in a passive refrigeration container at 5°C for 24 h before the cauda epididymal sperm was harvested (G3). Sperm harvest from the epididymides for G2 and G3 was performed by retrograde flushing of the caudal portion of the epididymus with a non-fat milk extender®. The recovered sperm was then cryopreserved using BotuCrio™ extender. Sperm motility parameters were analyzed by CASA, and apoptosis was estimated using epifluorescence microscopy for caspase activity and membrane phospholipid translocation. Samples were evaluated immediately (0 h) and 8 h after thawing.

3. Results and Discussion

At 0 h, no differences in sperm parameters were observed between treatments, but after 8 h, significant statistical differences were observed in sperm motility parameters and plasma membrane integrity between treatment groups. In addition, viable cells with no apoptotic signs were higher in G2 and G3, suggesting that epididymal sperm is less sensitive to the cold shock caused by sperm cryopreservation. These results indicate that freezing epididymal sperm under the conditions reported here is a good alternative to preserve the genetic material.

Footnotes

aBotuCrio™, Botupharma, Botucatu, Sao Paulo, Brazil.
bBotu-Semen™, Botupharma, Botucatu, Sao Paulo, Brazil.
cBotuContainer™, Botupharma, Botucatu, Sao Paulo, Brazil.

Research Abstract

NOTES