Available online at www.sciencedirect.com

ScienceDirect

Theriogenology 76 (2011) 252–260

Theriogenology

www.theriojournal.com

Evaluation of an animal protein-free semen extender for cryopreservation of epididymal sperm from North American bison (*Bison bison*)

S. Krishnakumar[a], D.P. Whiteside[b,c], B. Elkin[b,d], J.C. Thundathil[a,*]

[a] *Department of Production Animal Health, Faculty of Veterinary Medicine, University of Calgary, Calgary, AB, T2N4N1, Canada*
[b] *Department of Ecosystem and Public Health, Faculty of Veterinary Medicine, University of Calgary, Calgary, AB, T2N4N1, Canada*
[c] *Animal Health Centre, Calgary Zoo, Calgary, AB, T2E8K2, Canada*
[d] *Wildlife Division, Government of Northwest Territories, Environment and Natural Resources, Yellowknife, NT, X1A3S8, Canada*

Received 17 June 2010; received in revised form 17 December 2010; accepted 3 February 2011

Abstract

The objective was to evaluate the suitability of an animal protein-free semen extender for cryopreservation of epididymal sperm from the two subspecies of North American bison: plains (*Bison bison bison*) and wood (*Bison bison athabascae*) bison. Both cauda epididymides (from six plains and five wood bison) were minced and incubated in Sp-TALPH buffer for approximately 2 h at 37 °C to release actively motile sperm. Sperm suspensions were filtered, centrifuged and the sperm pellet from each bull was divided into two fractions and diluted either in egg yolk containing extender, Triladyl, or in an animal protein-free extender, Andromed, and equilibrated for 20 min at 37 °C. Thereafter, samples were chilled and cryopreserved. Frozen-thawed sperm were evaluated for motility (computer assisted sperm analysis), viability (SYBR 14 and propidium iodide), acrosome integrity (FITC conjugated PSA), cryocapacitation (tyrosine phosphorylation of sperm proteins as a biomarker), and fertilizing ability (in a heterologous IVF system). There was no significant difference for progressive motility, viability, and acrosome integrity between the two extenders for plains bison (36.8 ± 9.0, 60.5 ± 17.4, and 77.3 ± 4.6%; overall mean ± SD) as well as for wood bison (11.7 ± 8.1, 13.7 ± 5.6, and 73.4 ± 4.2%). Levels of tyrosine phosphorylation did not differ for sperm preserved in the two extenders for both subspecies, although an inter-bull variability in the response to tyrosine phosphorylation between extenders was suggested for plains bison. Fertilization percent did not differ significantly between extenders for plains bison (84.16 ± 9.92%, overall mean ± SD) and for wood bison (59.53 ± 19.99%). In conclusion, in the absence of significant difference between extenders in post-thaw sperm characteristics, we inferred that Andromed (animal protein-free) was suitable for cryopreservation of epididymal sperm from North American bison.
© 2011 Elsevier Inc. All rights reserved.

Keywords: American bison; Epididymal sperm; Cryopreservation; Semen extenders; Sperm analysis; Heterologous IVF

1. Introduction

There are two subspecies of the North American bison: wood (*Bison bison athabascae*) and plains (*Bison bison bison*) bison, with wood bison being classified as 'threatened' in Canada [1]. Organizations involved in wood bison conservation (e.g., the national Wood Bison Recovery Team) have supported research into the development and assessment of appropriate reproductive technologies to salvage bison genetics. Sperm collection, cryopreservation and use for IVF or AI are powerful technologies for conserving popula-

* Corresponding author. Tel.: +1 403 220 8244; fax: +1 403 210 3939.
E-mail address: jthundat@ucalgary.ca (J.C. Thundathil).

0093-691X/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.theriogenology.2011.02.001

tions of wild animals. Sperm cryopreservation facilitates movement of male genetics between populations and across borders, without the need for physically moving animals. Furthermore, it can be used for preserving the genetics of a male after death and is suitable for preserving the genetics of diseased animals, since the threat of disease transmission through this technology is very low [2].

Our laboratory is involved with developing reproductive technologies for salvaging the genetics of wood bison. Using plains bison as a model, we previously reported an efficient method for recovery and cryopreservation of epididymal sperm [3] in Triladyl, an egg yolk containing extender. Egg yolk or milk has conventionally been added to semen extenders. The role of these components in protecting sperm has not fully been understood, although several mechanisms have been proposed [4,5]. Low density lipoproteins in egg yolk and casein micelles in skim milk interact with and sequester bovine seminal plasma proteins which have been demonstrated to induce cholesterol and phospholipid efflux from sperm membranes, leading to membrane destabilization [5]. However, addition of egg yolk and milk contribute to variability among batches of extender, and increase the risk of disease transmission [6]. The use of an extender free of animal proteins decreases the risk of transmission of pathogens and viruses and would be preferred if found to efficiently preserve post-thaw sperm function. Andromed is a commercially available semen extender containing phospholipids derived from soybean extract. Sperm from European bison (*Bison bonasus*) and African buffalo (*Syncerus caffer*) have been successfully cryopreserved using egg yolk-free semen extender [7,8]. In the European bison, significantly more viable sperm with intact acrosomes were present in Triladyl, an egg yolk based semen extender, compared to an extender containing lipids from plant extract. However there was no significant difference in the fertilizing ability of sperm between the two extenders. For the North American bison, based on preliminary results, we concluded that Andromed, a synthetic semen extender, could be used for cryopreserving epididymal sperm from plains bison [3]. However, the fertilizing ability of such sperm has not been evaluated. The objective of the present study was to compare post-thaw characteristics and *in vitro* fertilizing ability of sperm cryopreserved in Andromed versus Triladyl, from both subspecies of the North American bison.

2. Materials and methods

Unless otherwise stated, all chemicals were purchased from Sigma-Aldrich (Oakville, ON, Canada).

2.1. Recovery and cryopreservation of epididymal sperm

Testes from plains bison (n = 6 bulls) were collected at a local abattoir, whereas testes of wood bison (n = 5 bulls) were collected from free-ranging animals, following an annual harvest for disease monitoring, conducted by the Wildlife Division, Government of Northwest Territories. Bison epididymal sperm were recovered and cryopreserved according to the procedure previously described [3], with minor modifications. Briefly, testes were recovered within 30 min after slaughter. Paired cauda epididymides from each bull were minced and transferred into tubes containing modified Tyrode's Hepes-buffered medium (Sp-TALPH; [9]) at 37 °C. Samples were held in Sp-TALPH medium at 37 °C for 2–3 h to allow actively motile sperm to swim out of the epididymides. Thereafter, samples with $> 50\%$ motility (visual assessment using $200 \times$ magnification) were filtered through sterile gauze and suspensions obtained were centrifuged ($500 \times g$) for 5 min. The sperm pellet was divided into two fractions; one fraction was extended in ~1 mL of pre-warmed animal protein-free semen extender (Andromed; Minitube, Ingersoll, ON, Canada), and the other fraction in ~1 mL of pre-warmed egg yolk containing extender (Triladyl; Minitube). Concentration of each fraction was determined using a hemocytometer, and adjusted to approximately 200×10^6/mL with the respective extender. Extended sperm were incubated in a water bath at 37 °C for 20 min. Tubes of extended sperm were then transferred to a beaker containing water at 37 °C and the beaker was placed in a fridge at 4 °C for at least 4 h for chilling and equilibration. Preparations with $>$ 50% motility (visual assessment using $200 \times$ magnification) were manually loaded into 0.5 mL straws and sealed with glass beads (Minitube). Filled and sealed straws were held in liquid nitrogen vapours for 15 min (approximately 2 cm above the surface of the liquid nitrogen in a styrofoam box) before plunging into liquid nitrogen. Cryopreserved sperm extended in Andromed or Triladyl were used for subsequent experiments.

2.2. Evaluation of post-thaw sperm characteristics

2.2.1. Motility

Sperm motility characteristics were evaluated for sperm cryopreserved in Andromed or Triladyl, using

Download English Version:

https://daneshyari.com/en/article/10893046

Download Persian Version:

https://daneshyari.com/article/10893046

Daneshyari.com