

Recovery and cryopreservation of epididymal sperm from agouti (*Dasiprocta aguti*) using powdered coconut water (ACP-109c) and Tris extenders

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Abstract

The objective was to compare the use of powdered coconut water (ACP-109c; ACP Biotecnologia, Fortaleza, CE, Brazil) and Tris extenders for recovery and cryopreservation of epididymal sperm from agouti. The caudae epididymus and proximal ductus deferens from 10 sexually mature agoutis were subjected to retrograde washing using ACP-109c (ACP Biotecnologia) or Tris. Epididymal sperm were evaluated for motility, vigor, sperm viability, membrane integrity, and morphology. Samples were centrifuged, and extended in the same diluents plus egg yolk (20%) and glycerol (6%), frozen in liquid nitrogen, and subsequently thawed at 37°C for 1 min, followed by re-evaluation of sperm characteristics. The two extenders were similarly efficient for epididymal recovery, with regard to the number and quality of sperm recovered. However, for both extenders, sperm quality decreased ($P < 0.05$) after centrifugation and dilution. After sperm cryopreservation and thawing, there were (mean $\pm$ SEM) $26.5 \pm 2.6\%$ motile sperm with 2.6 ± 0.2 vigor in the ACP-109c (ACP Biotecnologia) group, which was significantly better than $9.7 \pm 2.6\%$ motile sperm with 1.2 ± 0.3 vigor in Tris. In conclusion, agouti epididymal sperm were successfully recovered using either ACP-109c (ACP Biotecnologia) or Tris extenders; however, ACP-109c (ACP Biotecnologia) was a significantly better extender for processing and cryopreserving these sperm.

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Keywords: *Dasiprocta aguti*; Epididymis; Sperm; Cryopreservation; Coconut water

1. Introduction

Agoutis are neotropical terrestrial rodents in the family *Dasyproctidae*. They are distributed from southern Mexico to Argentina, including throughout Brazil [1]. Breeding agoutis in Brazil requires authorization from the Brazilian Institute for Natural Resources (IBAMA). However, commercialization of their meat remains to be widely explored [2], although they are hunted by the local population for historical and cul-

tural reasons [3]. Despite their wide geographical distribution and relative abundance, there are few reports regarding agouti sperm, including the physiology [4,5], and conservation [6].

Recovery, conservation, and fertilizing ability of epididymal sperm have been reported for several domestic species, including buffalo [7], dogs [8], horses [9], and cats [10]. Recent studies have also demonstrated that recovery and conservation of the epididymal sperm could be an alternative for wild animals, e.g., bison [11]. The potential use of epididymal sperm is attractive, especially following the death of a male. In that regard, epididymal sperm could be recovered postmor-

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tem, cryopreserved, and used for IVF when appropriate oocytes became available [12].

Choosing an appropriate extender is an important aspect of successful sperm cryopreservation. Tris-based diluents have been widely used for domestic mammals [13,14]. In addition, this buffering medium, in combination with egg yolk and glycerol, has also been used for cryopreservation of ejaculated [15] and epididymal [16] sperm of several wild species, but has apparently not been used for agouti. However, a milk-based extender and in natura coconut water were tested for cryopreservation of ejaculated agouti sperm [6].

Because coconuts are not universally available, powdered coconut water (ACP, ACP Biotecnologia, Fortaleza, CE, Brazil) was developed [17]. Although ACP was a suitable alternative for the conservation of the ejaculated sperm from several species [18,19], it has apparently never been used for recovery or conservation of epididymal sperm of any species. It is noteworthy that ACP is manufactured according to the sperm characteristics of the animals, with a registry number for each species. In that regard, ACP-109c (ACP Biotecnologia) was designated for use with agouti sperm. The objective of the present study was to evaluate the use of ACP-109c (ACP Biotecnologia) and Tris extenders for recovery and cryopreservation of epididymal sperm from *Dasyprocta aguti*.

2. Materials and methods

2.1. Animals

The procedures conducted in the present research were done in accordance with international animal care regulations. The animals belonged to the Centre of Multiplication of Wild Animals from UFERSA (Mossoró, RN, Brazil; 5°10'S, 37°10'W). The climate is typical semiarid, with an average annual temperature of 27°C. This center has approximately 150 agoutis, with an annual planned cull for population control.

Ten sexually mature male agoutis, with an average ($\pm$ SD) age and body weight of 30 ± 0.5 mo and 2.5 ± 0.2 kg, respectively, were used. These animals were isolated from females for 6 mo before the commencement of the study and maintained under a 12-h natural photoperiod. They were grouped and maintained in a covered paddock (4×5 m). The agoutis were fed a commercial rabbit ration, with 13% crude protein, 35% ether extract, 16% fiber, and 13% minerals. Fresh drinking water was available ad libitum.

2.2. Obtaining epididymal sperm

Animals were fasted for 12 h, restrained using a hand net, and premedicated with 15 mg/kg ketamine hydrochloride (Ketalar; Pfizer, São Paulo, Brazil) and 1 mg/kg xylazine hydrochloride (Rompun; Bayer, São Paulo, Brazil) given im. After 15 min, anesthesia was induced with sodium thiopental (Thiopentax; Cristalia, São Paulo, SP, Brazil) given iv, and the animals were subsequently killed with 1 mL/kg potassium chloride given iv. Immediately thereafter, the abdomen was opened and the testis (including the epididymis and proximal ductus deferens) were recovered. The carcasses were subsequently used for other experiments (morphology and meat science).

The testes and adnexa were examined to rule out any pathology and further washed in a physiologic salt solution. For each testis, the cauda epididymus, including 2 to 3 cm of the proximal ductus deferens, was dissected free from the testes and the surrounding connective tissue and blood vessels. Epididymal sperm were obtained by retrograde flushing, as previously described for the agouti [20]. A blunt 30-gauge needle connected to a 1-mL sterile plastic syringe was inserted into the ductus deferens. Each cauda epididymis was subjected to a retrograde flush with 0.5 mL of solution (at room temperature), and the expelled fluid collected in a 10-mL sterile plastic tube. As the epididymal ducts became distended with flushing media, stab incisions were done to improve recovery of the fluid. Both epididymides from the same animal were randomly washed using two solutions: the first epididymis was washed with powdered coconut water (ACP-109c; ACP Biotecnologia; 290 mOsm/L), obtained by the atomization process in a spray dryer and dissolved in ultrapure water; the second epididymis was washed with 3.028 g Tris-hydroxymethyl-aminomethane (Sigma-Aldrich Co., St. Louis, MO, USA), 1.78 g monohydrated citric acid (Sigma-Aldrich Co.) and 1.25 g D-fructose (Sigma-Aldrich Co.) dissolved in 100 mL ultrapure water (Tris; 295 mOsm/L). Sperm evaluation was done immediately after sperm washing.

2.3. Sperm evaluation

The volume recovered from each epididymis was measured with a micropipette, and the original flush volume (0.5 mL) was subtracted. Sperm concentration for each sample was determined using a Neubauer counting chamber, and the number of recovered sperm calculated. Sperm motility and vigor (strength of the sperm flagellum beating on a 0 to 5 scale) [21] were assessed immediately using light microscopy at $\times 100$

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