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Collection and evaluation of epididymal sperm in captive agoutis (*Dasyprocta aguti*)

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Abstract

The objective was to establish a protocol for the collection and evaluation of epididymal sperm in agoutis. Eight males (1–2 y old) underwent left orchidectomy and epididymal sperma were collected by retrograde flush. Average values were flush volume 32 μL, pH 6.9, sperm concentration 748 x 10^6 sperm/mL, with motility 86.5% and vigor 4.6. Viable sperm were present in all flush samples; 66% of sperm were alive, and 41.9% of sperm responded positively to the hypoosmotic test (using distilled water). There were 21.1% morphologically abnormal sperm, of which 2.0 and 19.1% were primary and secondary defects, respectively. The acrosome was intact in 99.5% of sperm. The sperm head was 4.89 $\pm$ 0.41 μm long and 3.13 $\pm$ 0.35 μm wide, with an area of 13.01 $\pm$ 2.01 μm^2. Midpieces were 5.33 $\pm$0.44 μm long and 0.98 $\pm$ 0.13 wide, sperm tails were 29.91 $\pm$ 2.29 μm, and overall sperm length was 40.12 $\pm$ 2.44 μm. In conclusion, epididymal sperm collection from agoutis was satisfactory; the collected sperm has the potential to be stored, facilitating development of other reproductive biotechnologies for this species.

Keywords: Epididymal sperm; Sperm morphometry; Agoutis

1. Introduction

Reproductive biotechnologies can be used for the management and preservation of domesticated and wild animals. In rodents, electroejaculation has been reported, but results have been variable [1–4]. It is necessary to remove the seminal vesicles and coagulating glands before electroejaculation in rats and mice, due to coagulation of the semen, which blocks the urethra. [1]. In *Dasyprocta leporina*, electroejaculation has also been described [4], but it is not an efficient method of semen collection in this species.

The collection of sperm from the cauda epididymis yielded sperm capable of undergoing an acrosome reaction, attaching to the zona pellucida, and fertilizing oocytes [5–7]. In rats [8] and in chinchillas (*Chinchilla laniger*) [9], this alternative method of obtaining sperm

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has been successful. To our knowledge, collection and evaluation of epididymal sperm from agoutis has not been reported. Therefore, the objective of the present study was to establish a protocol for the collection and evaluation of epididymal sperm in agoutis.

2. Materials and methods

2.1. Animals

Eight male agoutis (*Dasyprocta aguti,* Linnaeus, 1766) aged 1 to 2 y, and weighing between 1.8 to 2.4 kg, were used in this study. These animals were from the Centro de Multiplicação de Animais Silvestres (Multiplication Center of Forest Animals) (CEMAS) from the Universidade Federal Rural do Semi-Árido (UFERSA) (Federal Rural University of the Semiarid Region), scientific breeding center registered at IBAMA (Brazilian Environment and Natural Resources Institute) no. 1/24/92/0040-4, and introduced in the Núcleo de Estudos, Produção e Preservação de Animais Silvestres (NEPPAS) (Center for Study, Production and Preservation of Forest Animals) from the Universidade Federal do Piauí (UFPI) (Federal University of Piauí), in Teresina, a breeding center registered at IBAMA (number 02/08–618). Experimental protocols and animal care were approved by the research committee of the UFPI. The agoutis were allowed 1 mo to adapt prior to the study.

Agoutis were housed individually in 100 x 60 x 40 cm (length x width x height) cages and fed rodent food and fresh vegetables, with *ad libitum* access to water. They were exposed to a natural photoperiod (approximately 12 h light and 12 h dark throughout the year) [10].

2.2. Hemi-orchiectomy

The agoutis were fasted for 6 h prior to chemical restraint, then taken to the Laboratório de Pesquisas Morfológicas em Ciência Animal (Laboratory of Morphological Research in Animal Sciences) at UFPI, where collection and analysis of sperm was performed.

The anesthetic protocol was 5 mg/kg pethidine hydrochloride (Cristália, Itapira-São Paulo) given IM as a pre-anesthetic, approximately 10 min before induction of anesthesia. A combination of 35 mg/kg ketamine hydrochloride (Dopalen, Jacareí, SP, Brazil) and 1 mg/kg xylazine hydrochloride (Vetbrands, Paulínia, SP, Brazil) was given as a single IM injection. To maintain anesthesia, half the dosage of the same combination was subsequently used.

After induction of anesthesia, the inguinal region was shaved and surgically scrubbed before the left testis was removed [11]. Throughout the entire intraoperative period, the animals received fluid therapy with Ringer lactate (5 mL/kg/h IV), and rectal temperature and heart and respiratory rates were monitored. To prevent infection, 40,000 IU benzathine penicillin (Pencil B, Prodotti, São Paulo, SP) was given IM after anesthetic induction and repeated 48 h later.

2.3. Collection of epididymal sperm

Initially the testis-epididymis complex was separated and the cauda epididymis was rinsed with physiological saline at room temperature. Connective tissue covering the cauda epididymis was removed by careful dissection, as described [12], with care to avoid rupturing blood vessels or the epididymal duct. Both ends of the excised cauda were ligated to prevent loss of sperm. Thereafter, the cauda was kept in a vertical position, the lower ligature removed, and 0.2 mL of physiological saline was injected into the lumen (just below the remaining upper ligature), forcing sperm to flow through the cauda.

2.4. Evaluation of epididymal flush fluid and sperm

The volume of flush was measured with micropipettes, subtracting the volume of saline used for flushing the cauda. The color and the pH of the recovered fluid were evaluated by direct observation and pH strips (Macherey-Nagel, Germany), respectively.

The total motility and the speed of the sperm movement (vigor; scale 0–5) were analyzed under light microscopy, at magnifications of 100 and 400 X [13]. For assessment of sperm morphology and acrosomal integrity, Rose Bengal smears were prepared and analyzed under light microscopy (1000 X). Morphological sperm abnormalities were classified as primary and secondary [14]. For morphometric studies, normal sperm stained with Rose Bengal were analyzed in an Image Analyzer Computer Program Leica Qwin D-1000, Version 4.1 (Cambridge, UK) measuring length and width of the head and midpiece, head area, tail length, and overall sperm length. The percentage of living sperm was assessed on bromophenol blue-stained slides under light microscopy at 400 X magnification. Sperm concentration was calculated using a Neubauer chamber, and sperm membrane function was assessed with a hypoosmotic swelling test, performed with distilled water.

2.5. Statistical analyses

Descriptive statistics were generated with the StatView statistical software package (SAS Institute Inc., Cary, NC, USA). Data were reported as mean ± SD and range.