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RESEARCH ARTICLE

CRYOPRESERVATION OF GENETIC MATERIAL COLLECTED POST-MORTEM FROM MALE GRAY BROCKET DEER *MAZAMA GOUAZOUBIRA* FISCHER, 1814

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ABSTRACT

The objective of this study was to collect and cryopreserve spermatozoa collected post-mortem from the tail of the epididymis of a gray brocket deer (*Mazama gouazoubira*) in order to preserve the genetic material of the species. Spermatic cells of an adult animal were collected 5 hours after it was run over. The tails of the epididymides were dissected and sectioned, pressed to eliminate the cells, and washed in Dulbecco PBS. Physical and morphological analyses of the semen were based on routine methods. Semen was diluted 1:1 in Botubov® dilution medium in a final concentration of 810 million cells/mL. Diluted semen was cooled to 5°C and frozen at -196°C in 0.25 mL straws following a slow freezing curve. Thawing of the straws was carried out at 37°C/ 30 s. Total volume of semen was 3 mL, initial motility was 80%, vigor 3, and sperm cell concentration, 1.62 x 10^9 cells/mL, with 43% abnormal spermatozoa. After thawing, motility was 30% and vigor 3. It was concluded that gray brocket deer (*Mazama gouazoubira*) spermatozoa collected from the epididymis several hours after the death of the animal may be cryopreserved with good viability and used in in vitro assays.

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INTRODUCTION

The genetic material of wild animals may be lost by unexpected death or acquired reproductive incapacity. The creation of biobanks has an important role in the preservation and maintenance of genetic diversity (Strand *et al*., 2016). According to Keeley *et al*. (2012), cryopreservation of gametes together with artificial insemination may aid genetic management of captive animals and may serve as a source of genetic material to be introduced in wild populations.

Epididymal spermatozoa represent excellent sources of genetic material, given their quality and maturation stage. The recovery of spermatozoa from the epididymis of dead animals is an important technique in biodiversity preservation, as these gametes are able to undergo cryopreservation, and may be used in artificial insemination, *in vitro* embryo production, as well as other reproductive techniques (Turri *et al*., 2014).

The cryopreservation protocols for spermatozoa are species-specific and depend on the time and temperature of storage for the quality of the cells to be maintained. There are several sperm collection methods, such as compression of the tail of the epididymis, slicing, extrusion by air pressure, and retrograde washing of the vas deferens (Pablos *et al*., 2015), as well as directional freezing methods using liquid nitrogen vapor and immersion (Arav and Saragusty, 2016).

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In wild animals, this technique has been described in *Equus henionus onager* by Pablos *et al*. (2015), in *Capra pyrenaica* by Lópes-Saucedo *et al*. (2014), in *Sarcophilus harrisi* by Keeley *et al*. (2012), and in *Cervus elaphus hispanicus* and *Capreolus capreolus* by Martinez-Pastor *et al*. (2005).

In Brazil, the gray brocket deer (*Mazama gouazoubira)* is found from the south of the Amazon region to the Atlantic coastal area. This deer belongs to the order *Artiodactyla* and family *Cervidae*, and is an endangered species because of hunting and roadkill, as they cover long distances searching for females and foraging. It is a solitary species, only finding others individuals to mate (Duarte *et al*., 2012).

Therefore, the objective of this study was to post-mortem collect and cryopreserve spermatozoa from the tail of the epididymis of a gray brocket deer (*Mazama gouazoubira)* in order to preserve the genetic material of the species.

MATERIAL AND METHODS

Sperm cells came from an adult gray brocket deer (*Mazama gouazoubira)* that died after being run over in the Uberlandia region, Minas Gerais, Brazil. The animal was brought to the Teaching and Research Laboratory in Wild Animals at the Federal University of Uberlandia, arriving 5 hours after its death.

The testicles and epididymis were quickly removed, placed in plastic bags, and kept in ice at 5°C during the whole processing. The tails of the epididymides were dissected, sectioned longitudinally and transversally with a scalpel blade number 23 and pressed for spermatozoa to be released. After

that, epididymides were washed in modified Dulbecco PBS, and the liquid was recovered in a sterile flask. Physical analysis for motility (percentage) and vigor (0 to 5 scale) of the semen were carried out under a light microscope at 400x magnification. A Neubauer chamber was used to determine the number of cells per mL, and sperm morphology was carried out in a wet mount preparation using phase contrast microscopy to determine the percentage of abnormal cells (CBRA, 2013).

Semen collected was immediately diluted 1:1 in Botubov® dilution medium (Botupharma, Botucatu, Brazil). Solutions were mixed at room temperature to a final concentration of 810 million cells/mL. The sperm suspension with the diluent was placed in a beaker with water and then in a refrigerator at 5°C. The suspension was kept in equilibrium for two hours. After that, samples were placed in 0.25mL french straws (Minitub, Porto Alegre, Brazil) and frozen in a semen freezer model TK3000® (TK equipment's, Uberaba, Brazil). The freezing curve was: 0.5°C/min to 5°C; 5°C to -32°C in 15°C/min; -32°C to -120°C in 10°C/ min. In the end of the process, samples were dipped in liquid nitrogen at -196°C and were stored in a cryogenic bottle. Thawing was carried out at 37°C/ 30s in a WTA® semen thawer (WTA, Cravinhos, Brazil).

RESULTS AND DISCUSSION

In this study, epididymides were processed at about 5°C, similar to the temperature used in the study by Soler *et al.* (2003) and by Martinez-Pastor *et al.* (2005) in deer, and by Pablos *et al.* (2015), in onager. Cooling of the epididymides soon after collection is very important to preserve the quality of the spermatozoa pre- and post-freezing (Turri *et al.*, 2014); storage time should preferentially be no more than 24 hours (Strand *et al.*, 2016). From this moment on, there was a progressive reduction in quality (Martinez-Pastor *et al.*, 2005). However, epididymides of *Cervus elaphus hispanicus* kept at 5°C for up to 4 days kept their viability, with a little reduction in sperm motility (44.1±5.2%) in relation to the baseline values (57.6±1.6%) (Soler *et al.*, 2003).

Sperm recovery was performed by slicing of the tail of the epididymis, similar to the technique used by Soler *et al.* (2003), Martinez-Pastor *et al.* (2005), Keeley *et al.* (2012), and Pablos *et al.* (2015). This technique showed to be efficient, and the content collected had a great number of sperm cells. According to Hori *et al.* (2015) and Pablos *et al.* (2015) there is no difference between the quality of recovered sperm cells after collection and after thawing in both techniques.

Total volume of semen recovered was 3 mL and showed motility (MOT) of 80% and vigor 3 after removal from the epididymis. These results were similar to the ones reported by Lópes-Saucedo *et al.* (2014) in Iberian ibex (83.7% MOT; 3.6 vigor). However, they were lower than results by Soler *et al.* (2003) in deer (92% MOT; 3.8 vigor).

In the present study, a very high sperm concentration was observed, 1.62×10^9 cells/mL, and total concentration of 4.86×10^9 sperm cells. Lower total concentrations using the same technique were identified in Tasmanian devil (1.33×10^6) by Keely *et al.* (2012). However, Pablos *et al.* (2015) found values that were higher than the results of the present study (13.85×10^9 cells) in onager.

Variations in these physical parameters may be due to the characteristic of the species and/or reproductive period of the individual. In Brazil, gray brocket deers mate during the whole year, but they are more fertile in periods of high temperature and humidity (Duarte *et al.*, 2012), the period when the study was carried out.

The percentage of abnormal spermatozoa in the semen of the gray brocket deer was 43%. However, it is important to state that 30% of these sperm cells showed distal cytoplasmic droplets, a common defect in the epididymis and in young animals, such as the animal studied here. Besides these abnormalities, proximal cytoplasmic droplets (4%), tail defects (6%), and detached sperm heads (3%) were also observed. Sperm quality superior than the one in the present study was reported by Pablos *et al.* (2015) in onager with 93.7±0.9% normal sperm cells, and by Lópes-Saucedo *et al.* (2014) in Iberian ibex with only 3.7±0.4% abnormal cells. The good quality of spermatozoa from the tail of the epididymis was also reported by Arav and Saragusty (2016), who stated their good viability and fertility.

A good semen freezing protocol for most of the species should be carried out at 1 mm/s, at a slow gradient from +5°C to -50°C according to Arav and Saragusty (2016). The speed of freezing will determine how thawing will be performed (Soler *et al.*, 2003). The freezing curve used in this study was efficient in maintaining the characteristics of the sperm cells, following a slow reduction in temperature both in refrigeration and freezing, similar to the one used by Keely *et al.* (2012) in Tasmanian devil. Soler *et al.* (2003), Lópes-Saucedo *et al.* (2014), and Pablos *et al.* (2015) carried out rapid freezing for 7-15 minutes in nitrogen vapor (4-5 cm above the liquid), and then dipped the straws in nitrogen, which was also efficient.

In the present study, samples were thawed at 37°C/ 30 s. After thawing, motility was 30% and vigor 3. These values are considered good even for domestic animals (CBRA, 2013). The same protocol was used by Lópes-Saucedo *et al.* (2014) in Iberian ibex, with 55.5% MOT and 3.5 vigor, and Pablos *et al.* (2015) in onager with 27.5±4.3% MOT. According to Soler *et al.* (2003), cell viability may be widely affected by the thawing method. When different methods were tested, the highest semen fertility indices (69.7%) were obtained with samples thawed at 37°C/ 20 s.

The continuous decline in wild gray brocket deer populations (*Mazama gouazoubira*) shows the need to characterize the reproductive biology of this species and to develop complementary tools to aid in maintaining the genetic diversity of the populations.

CONCLUSION

The results of this study show that gray brocket deer (*Mazama gouazoubira*) sperm cells obtained from the epididymis several hours after the death of the animal may be frozen and thawed with good viability, and may be used in *in vitro* assays. This information increases the knowledge on sperm biology of this species and may be employed in the effective construction of genetic banks using dead animals.

References

Arav A, Saragusty J (2016) Directional freezing of sperm and associated derived technologies. *Animal Reproduction Science,* 169:6-13.

CBRA - Colégio Brasileiro de Reprodução Animal (2013) Manual para exame andrológico e avaliação de sêmen animal. CBRA, pp.103.

Duarte JMB, Reis ML (2012) Plano de ação nacional para a conservação dos cervídeos ameaçados de Extinção. Instituto Chico Mendes de Conservação da Biodiversidade, pp.66.

Hori T, Atago T, Kobayashi M, Kawakami E (2015) Influence of different methods of collection from the canine epididymides on post-thaw caudal epididymal sperm quality. *Journal Veterinary Medical Science*, 77(5):625-630.

Keeley T, McGreevy PD, O'Brien JK (2012) Cryopreservation of epididymal sperm colleted postmortem in the Tasmanian devil (*Sarcophilus harrissi*). Theriogenology, 78:315-325.

López-Saucedo J, Paramio MT, Fierro R *et al* (2014) Sperm characteristics and heterologous in vitro fertilization capacity of Iberian ibex (*Capra pyrenaica*) epididymal sperm, frozen in the presence of the enzymatic antioxidant catalase. *Cryobiology*, 68:389-394.

Martinez-Pastor F, Guerra C, Kaabi M *et al* (2005) Decay of sperm obtained from epididymes of wild ruminants depending on postmortem time. *Theriogenology*, 63:24-40.

Pablos MTP, Saragusty J, Santiago-Moreno J *et al* (2015) Cryopreservation of onager (E*quus hemionus onager*) epididymal spermatozoa. *Journal of Zoo and Wildlife Medicine*, 46(3):517-525.

Soler AJ, Garcia AJ, Fernández-Santos, MR *et al*. (2003) Effects of thawing procedure on post thawed *in vitro* viability and *in vivo* fertility of red deer epididymal spermatozoa cryopreserved at -196°C. *Journal of Andrology*, 24(5):746-756.

Strand J, Ragborg MM, Pedersen HS *et al*. (2016) Effects of post-mortem storage conditions of bovine epididymides on sperm characteristics: investigating a tool for preservation of sperm from endangered species. *Conservation Physiology*, 4:1-8.

Turri F, Madeddu M, Gliozzi TM *et al* (2014) Effect of testicle postmortem storage on goat frozen-thawed epididymal sperm quality as a tool to improve genebanking in local breeds. *Animal*, 8(3):440-447.