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Semen collection and evaluation of captive coatis (*Nasua nasua*)

[*Coleta e avaliação de sêmen de coatis* (Nasua nasua) *mantidos em cativeiro*]

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ABSTRACT

Semen samples (n=105) were collected through eletroejaculation from six adult male coatis (*Nasua nasua*) between January 2007 and December 2008 at Universidade Federal de Mato Grosso Zoo, Cuiabá, Brazil. Mean values were: volume (mL); concentration (sperm/mL); total motility (%); progressive sperm motility (scale, 0-5); live spermatozoa (%); acrossome integrity (%); primary defects (%); and secondary defects (%). There was high correlation between total motility and live sperm; total motility and progressive sperm motility; total motility and acrossome integrity; live sperm and progressive motility; live sperm and acrossome integrity and volume and concentration. The method for semen collection was considered safe and efficient. It can be used for the evaluation of breeding potential of coati in captivity and for the establishment of new assisted reproductive technology (ART) for threatened neotropical carnivores species.

Keywords: Procyonidae, semen, eletroejaculation, reproduction

RESUMO

Amostras de sêmen (n=105) foram coletadas por eletroejaculação em seis coatis (Nasua nasua) machos adultos entre Janeiro de 2007 a Dezembro de 2008 no Zoológico da Universidade Federal de Mato Grosso, Cuiabá, Brasil. Os valores mensurados foram: volume (mL); concentração (espermatozoides/mL); motilidade (%); vigor (escala: 0-5); espermatozoides vivos (%); acrossomas íntegros (%); defeitos primários (%) e defeitos secundários (%). Houve alta correlação entre motilidade e espermatozoides vivos, motilidade e vigor; motilidade e integridade de acrossoma; espermatozoides vivos e vigor; espermatozoides vivos e integridade de acrossoma; e volume e concentração. O método de colheita de sêmen foi seguro e eficiente podendo ser indicado para avaliação do potencial reprodutivo de coatis mantidos em cativeiro e para o estabelecimento de tecnologias de reprodução assistida (TRA) para espécies de carnívoros neotropicais ameaçadas de extinção.

Palavras-chave: Procyonidae, sêmen, eletroejaculação, reprodução

INTRODUCTION

Little research has been conducted on the reproductive characteristics of coati (*Nasua nasua*) (Barros *et al*., 2009; Lima *et al*., 2009); there are a few reports limited to the Procyonidae family (Beisiegel, 2001; Labate *et al*., 2001; Indrusiak *et al*., 2003; Franciolli *et al*., 2007).

Poor semen quality is a limiting factor for the majority wildlife animals in captivity. Inadequate environments and nutrition deficiencies are two specific factors that interfere with such reproduction (Mellen, 1991). Optimum nutrition and dietary husbandry is fundamental for the quality of the semen (Paz *et al*., 2006).

In addition, adult males of many seasonal breeding species have an annual cycle of testicular involution and recrudescence. In coatis, regression and recrudescence of testicular volume was associated with seasonal changes

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(Paz *et al*., in press). This strategy is advantageous to coatis, allowing males to minimize the energy expended on reproduction, thereby redirecting that energy to hunting and caring for the young (Lincon, 1981).

For the semen bank establishment, a safe semen collection method and the determination of semen characteristics according species and their seasonality is needed. Eletroejaculation is an effective and safe method for semen collection in wild animals. However, it is necessary to establish species-specific protocols (according to animal responses) and use an appropriate anesthetic procedure (Durrant, 1990).

The primary objective of the investigation was to establish a safe and effective technique for semen collection in male coati (*N. nasua*). A secondary objective was to establish physical and morphological characteristics of the coati (*N. nasua*) ejaculates.

MATERIALS AND METHODS

The study was conducted between January 2007 and December 2008 on six adult male (2-10 years old, body weight 5-7kg) coati from Federal University of Mato Grosso Zoo, Cuiabá City, Mato Grosso State, Brazil. There were 105 attempts to collect semen. The semen was collected once a month, with a minimum interval of 30 days. IBAMA (*Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis*) /Brazil and UFMT Bioetical Committee approved all the procedures in this study.

All the animals were housed together in outdoor pens without females. Coatis had ad libitum access to water and fruits (banana, papaya and mango), commercial dog food, egg, and fish or red meat (beef) were offered once a day. During the experimental period commercial vitamin and mineral supplement (Promater®/Vetnil, Brazil) were given daily (5mL/animal) mixed in the food.

Coatis' anesthetic protocols included tranquilizing associations or dissociative anesthetics sedatives. Animals were treated twice alternately in the twelve initial collections, with the following treatments: QX (Quetamine 10mg/kg and Xilazine 2mg/kg, IM); QXA (Quetamine 10mg/kg, Xilazine 2mg/kg and Atropine 0.04mg/kg, IM); QM (Quetamine 10mg/kg and Midazolam 0.5mg/kg, IM); QMAc (Quetamine 10mg/kg, Midazolam 0.5mg/kg and Acepromazine 0.1mg/kg, IM); Q(20)M (Quetamine 20mg/kg and Midazolam 0.5mg/kg, IM) and TZ (Tiletamine and Zolazepam 7mg/kg, IM). These anesthetic protocols were used with the objective of studying the cardiorespiratory response and characteristics of different dissociative anesthesia in a specific project, not related to this study. Afterwards, all animals were anesthetized with TZ treatment (12 collections).

Semen was collected using electroejaculation techniques. The Torjet 65C (Eletrovet®Brazil) electroejaculator was used in conjunction with a rectal bipolar electrode with three longitudinal copper bands 8.8cm in length and 0.2cm in diameter. The electrode was 1.8cm in diameter and 17cm long (Viana *et al*., 2007). The electrical stimuli were administered divided into three series (30:30:30) as follows: series 1 (ten stimuli at 200mA, ten at 250mA and ten at 300mA), series 2 (ten stimuli at 250mA, ten at 300mA and ten at 350mA) and series 3 (ten stimuli at 350mA, ten at 400mA and ten at 450mA) (Howard, 1993).

An aliquot of the ejaculate was placed on a microscope slide at 37°C, covered with a warm glass coverslip and examined at 400 x magnification. Total motility was assessed on a scale of 0 to 100% and progressive sperm motility on a scale of 0 to 5.

Sperm morphology and concentration were evaluated by fixing a semen aliquot (1:3 dilution) in a 10% formaldehyde saline solution. The concentration was evaluated using a Neubauer chamber under light microscopy at 400x magnification (Feldman *et al*., 1996). For sperm morphology determination, 100 cells per slide were counted under a light microscope at 1000x magnification and abnormalities were classified as primary or secondary defects according to Blom (1950).

To determine the live sperm proportion (%), one semen drop was mixed with one Eosin Y drop (Merck®), and to determine the acrossome integrity proportion (%), one semen drop was mixed with one Pope drop (Pope *et al*., 1991). A smear was prepared for each staining and 200

sperm cells were assessed under optical microscope at 1000x magnification.

Mean and Standard Deviation (SD) were determined for all data. The spearman correlation was determined between total motility and live sperm; total motility and progressive sperm motility; total motility and acrossome integrity; volume and concentration; volume and live sperm; volume and total motility; progressive sperm motility and volume; progressive sperm motility and concentration; live sperm and progressive motility; and live sperm and acrossome integrity (Statistic Analyses Program. 99 2th Ed. Stat Soft Inc.).

RESULTS

For the correlation analyses, a high positive correlation was found between total motility and live sperm (r=0.79, P<0.002); total motility and progressive sperm motility (r=0.97, P<0,0001); total motility and acrossome integrity (r=0.84, P<0.0005); live sperm and progressive sperm motility (r=0.8, P<0,01); live sperm and acrossome integrity (r=0.93, P<0.0001) and volume and concentration (r=0.76, P<0.003). None of the other correlations were significant.

Mean percentages of total and progressive motility had seasonal trends, similar to sperm concentration, with differences between seasons (P<0.0001, P<0.0004, and P<0.007, respectively). Total motility, progressive motility, and sperm concentration all peaked in the winter.

The semen-collection technique was highly efficient and 100% of the procedures produced an ejaculate. Urine contamination occurred in 15% of ejaculates. However, the semen quality differs according the season, making semen analysis impossible in the summer. Only 105 samples were analyzed in the total of 144 procedures.

Mean and SD of physical and morphological characteristics of the total ejaculates are presented in Table 1.

Table 1. Mean and SD of physical characteristics and sperm abnormalities in coatis (*Nasua nasua*) ejaculates (n=105) collected through electroejaculation, Cuiabá/MT, Brazil

End Point	Mean±SD
Volume (mL)	0.2±0.16
Total motility (%)	44.8±8.3
Progressive motility (0-5)	2.1±0.38
Concentration (x10^6 sperm/mL)	131.9±86.5
Live sperm (%)	66.6±7.7
Acrossome integrity (%)	72.9±10.3
Primary abnormalities (%)	21.5±7.9
Secondary abnormalities (%)	16.4±4.8
Total abnormalities (%)	37.9±9.8

DISCUSSION

Electroejaculation was a suitable method for semen collection in this species. The semen collection technique was efficient. It is noteworthy that coatis had no change in behavior or health, suggesting that the protocol used was clinically and ethically acceptable. The ability to collect semen samples from this species is an important factor in the development of assisted reproductive technologies.

Previous data has demonstrated that coatis are classified as seasonally breeding depending on location. In the South of Brazil the coatis' breeding season occurred between October and February (Indrusiak *et al*., 2003). According to Beisiegel (2001) the breeding season occurred between August-September and births between October-November in the South-east of Brazil.

Semen volume in the current study was 0.2±0.16mL. Despite the low ejaculate volume, sperm concentration was good (131.9±86.5x10^6 sperm/mL). Total sperm motility (44.8±8.3%) and progressive sperm motility (2.1±0.38) were high in the present study if compared to Viana *et al*. (2007) (22.5±38.62% and 1.5±2.38, respectively), but low when compared to Barros *et al*. (2009) (66.1±30.1% and 3.1±1.7, respectively), and Lima *et al*. (2009) (68% and 3.2, respectively). Distinct seasonal changes observed in these parameters had the highest values occurring during the winter, which corresponded to the breeding season for coatis at Pantanal/MT (Paz *et al*, in press).

In the study conducted by Barros *et al*. (2009) and Lima *et al*. (2009) semen collection occurred from February to October 2007, total sperm motility and progressive sperm motility found in the present study in the same period was 69.5±28.9% and 3.2±1.6, respectively. According to Viana *et al*. (2007) semen collections occurred from October/2006 to January/2007 justifying the low sperm motility and forward progressive sperm motility observed, suggesting the influence of seasonality in semen quality in animals living at Pantanal/MT.

In Panama, births in the white-nosed-coati (*Nasua narica*) population occurred between April and early May and parturition coincided with the period of highest availability of fruits (Smythe, 1970). In Mexico, births occurred by late June (Valenzuela, 1998), concurrent with the onset of the wet season, when water is not limited and the availability of arthropods reaches its maximum level (Lister and García, 1992). This is a common reproductive strategy, to increase the probability of survival of newborns, and occurs in many vertebrates inhabiting markedly seasonal environments.

The reproductive seasonality of this species did not seem to be correlated to the photoperiod; however, it was correlated to availability of resources.

The incidence of sperm abnormalities (37.9±9.8%) seemed relatively low. There is ample evidence in other species that diet, management, and environmental factors can affect semen quality (Meacham *et al*., 1963; Kanakara *et al*., 1984). Semen quality can also be substantially affected by the collection procedure or electroejaculation protocol (Morrel *et al*., 1996). Therefore, providing an optimal environment and collection procedure are critical to optimize semen quality. Furthermore, post-collection ejaculate handling avoids contamination and temperature changes, which is also important to maintain semen quality (Meacham *et al*., 1963; Kumar *et al*., 1984).

These data expand the knowledge of reproductive biology in male coatis, which is expected to assist in the development and implementation of reproductive management programs in captivity and free-living wild animals.

CONCLUSION

In conclusion, the electroejaculation protocol used was highly effective and safe, and a successful semen collection protocol considering the animals' seasonality is of critical importance.

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