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Description of semen characteristics from six-banded armadillos (*Euphractus sexcinctus*) collected by electroejaculation

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ABSTRACT

The aim of this study is to describe the characteristics of the semen from six-banded armadillos (*Euphractus sexcinctus*) collected by electroejaculation. Six mature males were physically restrained and electroejaculated twice for the collection of semen. Semen collected was immediately evaluated for appearance, volume, pH, sperm motility, vigor, morphology, percentage of live sperm and functional membrane integrity by light microscopy. Semen was obtained from all (100%) twelve attempts conducted for electroejaculation. Armadillos' semen had a white-translucent appearance, and great viscosity. Mean values obtained in analysis of the semen were: 353 ± 86 μl for volume, 9 for pH, $45 \pm 14 \times 10^6$ sperm/ml for concentration, 61 ± 7% motile sperm with 2 ± 0.2 for vigor, 55 ± 7% live sperm, 86 ± 2% morphologic normal sperm, and 46 ± 6% functional membrane integrity. In conclusion, semen from six-banded armadillos can be efficiently obtained by electroejaculation. The characteristics of semen collected by electroejaculation in six-banded armadillos provide background information that may be useful for assisted breeding programs in the members of the Xenarthra family.

1. Introduction

Six-banded armadillos (*Euphractus sexcinctus*) are wild mammalian natives of the Americas. They belong to the Xenarthra- or Edentata-order and to the Dasypodidae familly, and are characterized by an armor comprised of six to eight motile bands that cover the body, and a conic and flat head (Deem and Fiorello, 2002). They live in a variety of habitats, including semi-arid grasslands, brush land, forests, moist lowlands, savannahs, and riparian regions. Insects constitute the largest part of their diet, although other invertebrates, small rodents, lizards, snakes, carrion, fruits, and plant material are also consumed (Deem and Fiorello, 2002). Armadillos are considered primarily as a species with lonely habitation, except during the breeding season (Desbiez et al., 2006). The reproductive biology of this species has not been completely elucidated, but armadillos reach sexual maturity between 6 months (Talmage and Buchanan, 1954) and 2 years of age (Oliveira et al., 2007).

Many communities hunt these animals indiscriminately and use them as food supplement; this practice contributes to the reduction in the numbers living in their natural habitat. Moreover, the development in agriculture and reduction of protected areas over the past decades are responsible for precious loss of habitat for all the Endetata species (Fallabrino and Castiñera, 2006).

Knowledge of the reproductive characteristics of a species is important for applying biotechniques aimed at their preservation (Silva et al., 2004). The objective of the present study was to describe the characteristics of

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Table 1
Semen characteristics (means ± SEM) from six-banded armadillos (*Euphractus sexcinctus*) collected by electroejaculation ($n = 2$ ejaculates/male).

Characteristics	Male 1	Male 2	Male 3	Male 4	Male 5	Male 6	Average
Volume (μl)	300 ± 200[b]	388 ± 112[a,b]	146 ± 46[b]	80 ± 20[b]	900 ± 100[a]	305 ± 65[b]	353 ± 86
Sperm concentration ($\times 10^6$ ml^{-1})	37 ± 1	19 ± 14	20 ± 15	32 ± 20	61 ± 41	100 ± 70	45 ± 14
Motility (%)	55 ± 35	68 ± 27	68 ± 17	45 ± 5	75 ± 25	55 ± 15	61 ± 7
Vigor (0–5)	1.5 ± 0	2.5 ± 0.5	2.5 ± 0.5	2 ± 0	1.5 ± 0.5	1 ± 0	2 ± 0.2
Live sperm (%)	74 ± 15	59 ± 36	31 ± 19	50 ± 15	60 ± 10	57 ± 17	55 ± 7
Functional membrane integrity (%)	42 ± 29	70 ± 10	49 ± 20	54 ± 5	34 ± 6	31 ± 9	46 ± 6
Acrosomal integrity (%)	98 ± 1	99 ± 0.5	99 ± 0	99.5 ± 0.5	99.5 ± 0.5	99 ± 1	99 ± 0.3
Normal sperm morphology (%)	80 ± 10	90 ± 1	89 ± 1	90 ± 0	87 ± 6	80 ± 11	86 ± 2
Primary defects (%)	2 ± 1	2 ± 2	1.5 ± 1	1.5 ± 1.5	3 ± 1	2 ± 1	2 ± 0.5
Secondary defects (%)	18 ± 12	8 ± 1	9 ± 1	8 ± 2	10 ± 5	17 ± 12	12 ± 3
Total defects (%)	20 ± 10	10 ± 2	10 ± 1	10 ± 0	13 ± 6	19 ± 11	14 ± 2

[a,b]Indicate significant differences among individual males ($P < 0.05$).

the semen collected by electroejaculation from six-banded armadillos.

2. Materials and methods

2.1. Animals

Six mature male six-banded armadillos were used in this experiment that was conducted from August 2007 to March 2008. The animals weighed between 3 and 5 kg, and were kept under 11 h natural photoperiod conditions. They were housed in individual cages belonging to the Zoo-botanic Park Onélio Porto at Mossoró, located in Brazil's semi-arid region (5°10′S–37°10′W; temperature range, 27–29 °C). They were fed once a day with dog chow pellets and had free access to water.

2.2. Collection of semen

The animals were subjected to two sessions for the collection of semen, with a 1-month interval between sessions, in a total of 12 procedures. The animals were placed in a laterally recumbent position and the pubic region was cleaned. The electroejaculation procedure followed was the same for both treatments and lasted approximately 15 min. Semen collection was attempted with an electroejaculator (Eletrojet®, Eletrovet, São Paulo, SP, Brazil) connected to a 12 V source. There were three stimulation cycles, with a 5 min interval between successive cycles. The first cycle was consisted of 10 stimuli of 2, 3, and 4 miliampere (mA), in succession; the second was 10 stimuli of 3, 4, and 5 mA, sequentially; and the third was 10 stimuli of 5 and 6 mA (Wildt et al., 1983). If an animal did not ejaculate, a 15 min period of rest was provided and the procedure was immediately repeated. The electroejaculator probe measured 12.5 cm, but only ~8 cm was inserted into the rectum of each armadillo. Semen was collected in plastic tubes and immediately evaluated.

2.3. Evaluation of semen

Micropipettes varying from 5 to 200 μl were used to measure the volume of the semen collected and its appearance was examined. The pH was determined using pH-indicator strips (Neutralit®, Merck, Bucharest, Romania). Percentage of motile sperm and rate of sperm movement (vigor) were assessed immediately using light microscopy (Eclipse E200, Nikon, Melville, NY, USA) under 100× and 400× magnification. Bengal Rose (Sigma, Sigma–Aldrich, São Paulo, SP, Brazil) stained smears were prepared with 5 μl of diluted semen to evaluate sperm morphology and acrosomal integrity using light microscopy (1000×), counting 200 cells per slide (Yubi et al., 1987). Sperm morphologic abnormalities were classified as primary and secondary (Oettlé, 1993). Percentage of live sperm was established by analyzing a slide stained with brome-phenol blue (Sigma, Sigma–Aldrich, São Paulo, SP, Brazil) under light microscopy (400×), counting 200 cells per slide (Derivaux, 1980). Following initial assessment, a 10 μl semen aliquot was diluted in 10% buffered formalin (1 ml) and sperm concentration (sperm $\times 10^6$ ml^{-1}) was determined using a Neubauer counting chamber. For the evaluation of sperm membrane function, a hypo-osmotic swelling test was performed immediately after the collection of semen, using distilled water (0 mOsm/l) as the hypo-osmotic solution (Quintela et al., 2004); spermatozoa presenting swollen coiled tails were considered as functional sperm membrane.

2.4. Statistical analysis

The research committee of the Universidade Federal Rural do Semi-Árido, Mossoró, Brazil, approved the experimental protocols, and animal care procedures adopted. Statistical analyses were carried out using SigmaStat 3.5 (Systat Software Inc., San Jose, CA, USA). Semen characteristics are presented as mean values ± SEM. Data were checked for normality by Kolmogorov–Smirnov test with Lilliefor's correction, and for homoscedasticity by Levene's test. Data for volume, sperm motility, normal morphology and acrosomal integrity were compared among individual animals by Tukey's test. Data for sperm vigor, concentration, live sperm percentage, functional membrane integrity and morphological defects were ArcSin transformed and also compared by Tukey's test. The results were considered significant when $P < 0.05$.

3. Results

All the attempts for the collection of semen were successful, representing an efficiency of 100%, but we must point out that in four attempts (33.3%), animals ejaculated