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RESEARCH ARTICLE

ASSISTED REPRODUCTION IN ALLIGATORS: PHYSICAL AND MORPHOLOGICAL CHARACTERIZATION OF SPECTACLED CAIMAN SEMEN *CAIMAN CROCODILUS* LINNAEUS, 1758

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ABSTRACT

The objective of this study was to understand the physical and morphological characteristics of spectacled caiman semen *(Caiman crocodilus)*. Twenty-five free-living caimans from the state of Mato Grosso, Brazil (Amazon region) were used. Semen was collected by electro ejaculation under physical restraint. For the physical analysis of sperm cell motility and vigor, a drop of semen was placed on a glass slide and covered with a coverslip for optical microscopy. Semen concentration was determined in a Neubauer chamber and sperm morphology was determined by wet-mount preparation for phase-contrast microscopy. The volume of semen ranged from 200 to 500 µL; it was milky white; motility ranged from 80 to 90%; and mean vigor was from 2 to 4. Mean spermatozoon concentration in semen was 2.14 ± 1.98 billion/ml. As for sperm cell morphology, they were filiform with a pointy end and s-shaped heads; total length was 80.98 ± 1.29 µm. Percentage of sperm abnormalities was 14.75 ± 1.55%. Spectacled caiman semen showed optimum physical and morphological characteristics, with adequate quantity and quality, indicating high fertility rates. There are no definitions of ideal reproductive parameters for the species, and further studies are necessary on these reptiles.

INTRODUCTION

Caimans are reptiles that belong to the subclass Archosauria, order Crocodilia, family Alligatoridae, and subfamily Alligatorinae . Six species are found in Brasil: *Melanosuchus niger*, *Paleosuchus palpebrosus*, *Paleosuchus trigonatus*, *Caiman crocodilus*, *Caiman yacare* and *Caiman latirostris* (SBH, 2017).

The genital system of the male caiman is made up of two round testicles located close to the medial edges of the kidneys, in the dorsal part of the body, and sperm production gradually increases with the number of seminiferous tubules and the size of the animal (Bassetti, 2006). The system of ducts is formed by the *rete testis*, efferent ducts, epididymis, and vas deferens, which are responsible for the production and/or modification of the seminal fluid. The vas deferens is wide in diameter and it is where spermatozoa accumulate; it is also located toward the front of the body, joining the phallus on the floor of the cloaca (Guerrero *et al.,* 2004). Environmental factors such as temperature and water level are determinant for gonad activity, whose dimensions and histological appearance are influenced by seasonal effects, as the testicles show their largest size towards the middle of the reproductive season (Guillette and Milnes, 2000).

Researchers are paying attention to alligator reproduction, as it is the base for the management of these animals both in natural conditions and in captivity. The analysis of semen characteristics is extremely important in the evaluation of reproductive potential in males, involving physical (volume, color, motility, vigor and concentration) and morphological characteristics of the spermatozoa (abnormalities of the head, midpiece and tail) (CBRA, 2013). In reptiles, there are some reports on semen analyses: in iguanas by Healy and Jamieson (1994) e Zimmerman *et al*. (2013); in lizards by Teixeira *et al.* (2002); in snakes by Innocenti *et al.* (2006), Tourmente *et al.* (2007), and Zacariotti and Guimarães (2010); in turtles by Tanasanti *et al.* (2007); and in crocodiles by Romero-Solórzano *et al.* (2010), and Johnston *et al.* (2014 a,b).

Sperm cells abnormalities are associated with infertility in most species analyzed, given the important role of these cells in fecundation. In domestic mammals, birds, and fish, semen analysis have been classified by the Colégio Brasileiro de Reprodução Animal (CBRA, 2013) and, in crocodilians, reports only involve *Crocodylus intermedius* (Romero-Solórzano *et al.*, 2010) and *Crocodylus porosus* (Johnston *et al.*, 2014 a,b).

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Thus, given the lack of information on the semen profile of alligators, the objective of this study was to present the physical and morphological characteristics of spectacled caiman sperm cells (*Caiman crocodilus*).

MATERIAL AND METHODS

The study was carried out in free-living spectacled caimans (*Caiman crocodilus*) from Boa Esperança farm, located at 14°46'47,8"S, 51°32'50,9"W, 265 m above the sea level, in the city of Araguaiana, Mato Grosso, Brazil. Collection of semen was carried out in August, when mean temperature of the region ranges from 18 to 33°C and precipitation is about 10 mm (INMET, 2017), period with more reproductive activity of this specie. This study was licensed by Instituto Chico Mendes de Conservação da Biodiversidade, license number 45947-2. The study was authorized by the Ethics Committee in Animal Use from Universidade Federal de Uberlândia, Minas Gerais, Brazil, protocol number 112/2014.

The semen of 25 animals that measured more than 1.0 m in snout-vent length. Semen collected by electroejaculation and was performed with a portable electroejaculator with a maximum amperage of 12 volts (Autojac - Neovet®-Brazil), connected to a probe (18 x 2 cm) with 3 electrodes turned to the ventral region of the animal. The probe was introduced in the cloaca after exposure of the phallus. Five successive 2-second electric stimuli of 30 mA were performed, followed by 80 mA stimuli for 5 seconds, with a 5-minute interval between each session. All semen produced by the animals was collected. Preparation of the samples for the physical and morphological analyses was carried out according to the guidelines of the Colégio Brasileiro de Reprodução Animal (CBRA, 2013).

For the analysis of semen motility and vigor, a drop of semen was placed on a glass slide, covered with a coverslip, and analyzed under an optical microscope for progressive sperm cell motility (in percentage), and movement vigor (ranging from 0 to 5). Semen concentration was measured in a Neubauer chamber and sperm morphology was assessed by wet-mount preparation (one drop of semen in a glass slide covered by a coverslip) by phase-contrast microscopy, with assessment of the percentage of abnormalities in individual cells (CBRA, 2013). The morphological characteristics of the cells were observed in a glass slide stained by May-Grunwald-Giemsa, under a light microscope at 1,000 X magnification.

RESULTS AND DISCUSSION

Semen volume of spectacled caimans (*Caiman crocodilus*) ranged from 200 to 500 µL, and the liquid was milky white. The volume collected in this study was much larger than the one obtained by Larsen *et al*. (1992) in *Caiman latirostris*. This author reported no more than 100 µL after aspiration of the ejaculatory sulcus. In crocodiles, volumes collected were larger than in caimans, and Romero-Solórzano *et al*. (2010) reported 1-2 ml of semen, Johnston *et al*. (2014 a), 0.91 ± 0.16 ml, and Johnston *et al*. (2014 b), 1.4 ± 0.3 ml. These differences may be explained by the size of the animals and of their testicles, besides the individual response to the stimulus in semen collection.

Semen motility ranged from 80 to 90%, and mean vigor from 2 to 4 (mean 3), similar to the results from Romero-Solórzano *et al*. (2010) in *Crocodylus intermedius* (70 to 80%). In *Crocodylus porosus,* Johnston *et al*. (2014a) found lower motility and vigor, with means of 50.7 ± 4.2 and 2.6 ± 0.2, respectively, whereas Johnston *et al*. (2014b) reported means of 63.4 ± 3.2 for motility, and vigor of 4.0 ± 0.2. Similar results for motility (78%) also were verified in *Iguana iguana* by Zimmerman *et al*. (2013). Sperm cells motility and vigor are the most important analyses in semen, as they translate the viability of the spermatozoa.

Mean sperm cell concentration in semen was 2.14 ± 1.98 billion (0.63 - 5.9 x 10^9). Variations in concentration may be due to the quality of the ejaculate, age of the animal and reproductive activity. A wide variation, such as this, has also been reported in crocodiles by Romero-Solórzano *et al*. (2010), who observed concentrations of 5.2 - 20 x 10^9, Johnston *et al*. (2014a), with 0.35 - 4.0 x 10^9 (mean 2.29 ± 0.26), and Johnston *et al*. (2014b), with 2.5 - 4.2 x 10^9 (mean 3.4 ± 0.2). This variation may be expected, as in both this study and in others, adult animals of different ages and at different moments of the reproductive period were used.

Sperm cells of spectacled caimans (*Caiman crocodilus*) are filiform with a pointy end and s-shaped heads, as also observed by Tanasanti *et al*. (2007) in turtles, Zacariotti and Guimarães (2010) in snakes, and Jamieson *et al.* (1997) and Gribbins *et al*. (2011) in crocodiles (Figure 1A). Mean spermatozoon dimensions were: head, 20.09 ± 0.85 µm; midpiece, 2.40 ± 0.16 µm; and tail, 58.49 ± 0.29 µm; total mean length was 80.98 ± 1.29 µm (Figure 1B).

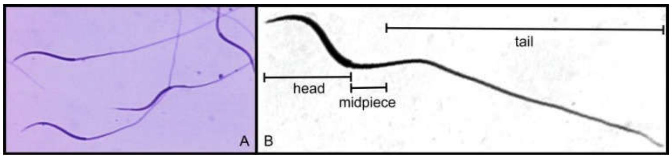

Figure 1 Photomicrograph of spectacled caiman (*Caiman crocodilus*) spermatozoa. A) Cell morphology; B) Parts of the spermatozoon. May-Grunwald-Giemsa staining, magnification 1.000x.

Dimensions recorded in caiman spermatozoa were similar to those obtained in crocodiles by Jamieson *et al*. (1997), who reported heads of 16.30 µm, midpieces of 4.80 µm, and tails from 49 to 61 µm, and by Romero-Solórzano *et al*. (2010) who reported heads of 19.09 ± 1.32 µm, and tails of 53.31 ± 3.56 µm. Gribbins *et al*. (2011) found *Alligator mississippiensis* spermatozoa showing total length of 97 ± 2.3 µm, longer than those of alligators in this study. Other reptiles, such as lizards and fresh water turtles (*Mauremys caspica*) showed longer spermatozoa compared with alligators, with total length of 54.50 µm to 75.58 µm according to the lizard subspecies (Teixeira *et al*., 2002), and 50 µm in turtles (Al-Dokhi *et al*., 2007). Iguanas showed large cells, around 146 µm (Healy and Jamienson, 1994). According to Morrow and Gage (2001) this variation in spermatozoon morphometry is associated with its function in the environment and the mating pattern of the species, as they have evolved to function in an inhospitable environment, but with optimum capacity to fertilize e compete in the environment.

The percentage of morphologically normal spermatozoa was 85.22%. There is no classification of defects proposed for reptiles, mainly for alligators, only for domestic animals (CBRA, 2013). The total of cells with head, midpiece, and tail abnormalities in the semen of spectacled caimans was 14.73 ± 5.36 %, with six types of defects: folded tails (4.73 ± 3.72 %), coiled tails (1.35 ± 1.35 %), abnormal head shapes (3.50 ± 2.67 %), midpiece defects (3.08 ± 1.97 %), normal isolated heads (1.23 ± 1.17 %), and strongly coiled or folded tails (0.85 ± 1.61 %).

Figure 2 shows the total number of morphological defects observed in sperm cells of spectacled caimans, and Figure 3 shows the different types of morphological abnormalities observed in spermatozoa.

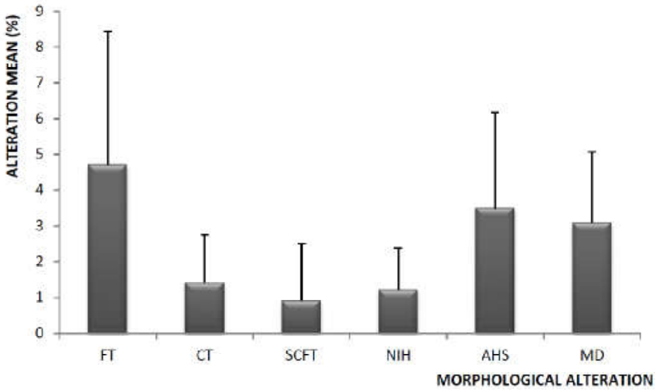

Figure 2 Morphological changes observed in sperm cells of spectacled caimans (*Caiman crocodilus*). FT - folded tail, CT - coiled tail, SCFT - strongly coiled or folded tail, NIH - normal isolated head, AHS - abnormal head shape, MD – midpiece defects.

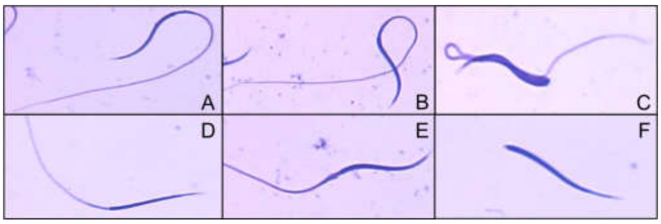

Figure 3 Photomicrographs of spermatozoa with morphological changes in spectacled caimans (*Caiman crocodilus*). A, B and C) Tail defect; D) Abnormal head shape; E) Midpiece defect; F) normal isolated head. May-Grunwald-Giemsa staining, magnification 1,000 x.

The number of spermatozoa that showed morphological defects in spectacled caimans in the present study was similar to the results obtained in crocodiles by Romero-Solórzano *et al*. (2010), who reported values between 13% and 29.5% for primary and secondary sperm cell abnormalities, and with the greatest number of cases involving midpiece defects (23.2%), followed by isolated heads (6.3%). Johnston *et al*. (2014a) reported much higher values in salt water crocodiles (*Crocodylus porosus*) with only 51.2% ± 5.1 of morphologically normal cells, and the most prevalent defect being cytoplasmic droplets (26.9%). In the same species, Johnston *et al*. (2014b) reported 86.2% ± 2.2 normal cells. Considering the reduced percentage of sperm cells defects, it may be inferred that spectacled caimans (*Caiman crocodilus*) used in this study have high quality semen, and it is expected that they present high fertility rates, as well.

CONCLUSIONS

Sperm cell morphology in spectacled caimans (*Caiman crocodilus*) is similar to that of other reptiles, with little differences in the shape of the heads. The semen collected demonstrated to have optimum physical and morphological characteristics, as well as good quality and quantity, showing indication of high fertility rates.

There are few reports on alligator reproduction, and the ideal reproductive parameters for the species are still undefined. Because of that, further studies are necessary on these reptiles.

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