Semen collection techniques for Spectacled Caimans

Caiman crocodilus (Linnaeus, 1758)

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Abstract. The objective of this study was to develop in vivo and post-mortem semen collection techniques for spectacled caimans (Caiman crocodilus). Twenty five free-living adults from the Brazilian Amazon region were used. Post-mortem collection was carried out in two animals that were euthanized with an overdose of propofol and potassium chloride 15% (> 10 mg/kg). The vas deferens were isolated and washed with saline solution slowly injected caudocranially, with recovery of the contents cranially in plastic tubes. Living animals were restrained by tying their limbs and mouths and were placed in dorsal decubitus. Collection was carried out by electroejaculation and massage of the base of the phallus. Post-mortem collection yielded samples with adequate number of cells, but high content of mucus. In vivo collection yielded 200 to 500 µL of semen with high numbers of motile spermatozoa, with electroejaculation being the most efficient technique. The three techniques were efficient and useful for studies of reproduction in caimans.

Keywords: Andrology, reproduction, reptiles

Introduction

Assisted reproduction in reptiles is still little explored, and there is little information on methods for semen collection, analysis, and freezing (Swanson, 2006; Zacariotti et al., 2007). In particular, the aggressiveness of crocodilians poses many complications for semen collection from living individuals, and electroejaculation after sedation or restraint has become the most common technique used in free-living or zoo reptiles (Durrant, 1990). In studies of crocodilian fertility, it is necessary to develop efficient semen collection techniques for artificial insemination, genetic management of captive animals, and breeding of endangered species (Johnston et al., 2014). Semen has been successfully collected from several species of crocodilians. For example, Larsen and Cardeihac (1984) carried out post-mortem semen collection from Alligator mississippiensis by vas deferens compression. Larsen et al. (1992) collected semen from Caiman latirostris by aspiration and scraping using a penile sulcus catheter. Johnston et al. (2014) collected Crocodylus porosus semen from sedated animals using manual massage of the final portion of the vas deferens and collecting semen from the ejaculatory sulcus. Non-invasive methods such as hormonal monitoring, semen banks, and artificial insemination have been used for genetic manipulation of ex-situ populations in order to restore wild populations, such as the work carried out with lizards in New Zealand (Molinia et al., 2010). Developing techniques for semen collection in alligators is also very important in order to produce samples that can be used in assisted reproduction.

Spectacled caimans are found from southern Mexico (Chiapas) through Central America into northern Peru, Bolivia, and Brazil, in the Amazon, Orinoco, Araguaia, and Tocantins river basins. This species is relatively small, with males reaching 2.0 to 2.5 m in length and females reaching 1.4 m (ICMBIO, 2017). Environmental factors such as temperature and water level affect 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 (Thorbjarnarson, 1994; Guillette and Milnes, 2000; Coutinho et al., 2005). Social interaction and vocalization occur during the reproductive period, mainly in the first hours of the morning, when the temperature is milder. Courtship and mating takes place...
The objective of this study was to develop \textit{in vivo} and \textit{post-mortem} semen collection techniques in spectacled caimans (\textit{Caiman crocodilus}).

\textbf{Material and Methods}

This study was carried out in free-living \textit{Caiman crocodilus} from Boa Esperança Farm (14°46’47,8”S, 51°32’50,9”W, elevation 265 meters) in Araguaiana, Mato Grosso, Brazil. The collections were carried out in August, when mean temperature ranges from 18 to 33 °C, and precipitation is very low, about 10 mm (INMET, 2017). Semen was collected from 25 animals measuring > 1.0 m long. The animals were kept restrained for a maximum of 20 minutes and were released right after the collection. The study was authorized by the Ethics Committee in Animal Use from Federal University of Uberlandia, Minas Gerais, Brazil, protocol number 112/2014. This study was licensed by Instituto Chico Mendes de Conservação da Biodiversidade, license number 45947-2.

For the post-mortem semen collection two animals were euthanized by overdose with propofol (> 10 mg/kg) associated with potassium chloride 15% (5 mL), and the reproductive tract was removed. The vas deferens were isolated and washed with 1.0 mL of saline.

\textbf{Figure 1.} \textit{Post-mortem} semen collection in \textit{Caiman crocodilus} by washing the vas deferens. Whole vas deferens (arrows); catheterized vas deferens (needles).

\textbf{Figure 2.} \textit{In vivo} semen collection in \textit{Caiman crocodilus} using electroejaculation. A) Phallus exposure; B) introduction of the probe in the cloaca; C) stimulation; D) erection; E) beginning of ejaculation; F) semen collection.
of 0.9% saline solution. The solution was slowly injected caudocranially (from cloaca to testis) using a hypodermic syringe, and the contents were recovered in 1.5 mL plastic tubes (Fig. 1).

Living animals were restrained by tying their limbs and mouths and placed in dorsal decubitus. Collection was carried out by electroejaculation or massage of the base of the phallus. All collections were carried out with physical restraint alone, without sedation. The cloaca of the animal was washed with 0.9% saline solution to remove any feces or urates before the collection. Afterwards, the region was dried with a paper towel and this was repeated if needed during the collection.

Semen collection by electroejaculation (Fig. 2) was performed with a portable electroejaculator operating between 20 and 60 Hz, and 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. The number of sessions varied for each animal, but the number of sessions was never higher than three. The ejaculate was collected from the tip of the ejaculatory sulcus of the phallus in a 1.5 mL plastic tube.

Collection by massage of the base of the phallus (Fig. 3) was based on a technique similar to the one described by Johnston et al. (2014) for saltwater crocodiles (Crocodylus porosus), but without sedating the animals (due to their smaller size) and by placing them in dorsal decubitus. The phallus was exposed from the proctodeum by placing a gloved finger in the cloaca and carefully pulling it outside. After that, a finger was placed behind the base of the phallus in the urodeum, and the final portion of the vas deferens was massaged repeatedly in the proximal-distal direction, beginning in the base of the phallus. Semen was collected from the tip of the ejaculatory sulcus using a 1 mL syringe.

For the analysis of semen motility and vigour, a drop of semen was placed on a glass slide, covered with a coverslip, and analysed under an optical microscope for progressive sperm cell motility (in percentage), and movement vigour (ranging from 0 to 5). Semen concentration was measured in a Neubauer chamber.

Results and Discussion

Both in vivo and post-mortem techniques used in the collection of semen samples were efficient and yielded large numbers of sperm cells. Semen collection in living animals yielded samples ranging from 200 to 500 µL with a milky white colour. Semen motility ranged...
from 80 to 90%, and mean vigour from 2 to 4 (mean 3). Mean sperm cell concentration in semen was 2.14 ± 1.98 billion (0.63 - 5.9 x 10⁶), and this variation in concentration may be due to the quality of the ejaculate, age of the animal, and reproductive activity.

Collection by massage of the base of the phallus resulted in a slightly smaller volume than collection by electroejaculation. The volume of semen obtained in this study was greater than that obtained by Larsen et al. (1992) in Caiman latirostris. These authors recovered no more than 100 µL after aspiration of the ejaculatory sulcus. On the other hand, Johnston et al. (2014) obtained about 910 mL of highly viscous semen from Crocodylus porosus > 1.8 m in length. In other reptiles, the amount of collected semen is lower than in caimans and the colour of the semen is different. In green iguanas (Iguana iguana) the semen colour can be clear, white or tan, and the average amount is 20 µL (Zimmerman et al., 2013). In corn snakes (Pantherophis guttatus), the semen colour varies from white to tan, with average amount of 10 µL (Fahrig et al., 2007). According to Zimmerman et al. (2013), the small amount of semen produced by these reptiles is due to the absence of auxiliary glands in these species, but this is compensated for by the high concentration of spermatic cells and favoured by the short distance that the spermatozoa have to cover in the female’s reproductive tract.

Electroejaculation in non-sedated Caiman crocodilus was efficient at producing good quality samples for semen analysis. The stimulus intensity was higher than that used for iguanas by Zimmerman et al. (2013) because, as proposed by Platz et al. (1980), some reptiles require maximum electric stimuli (verified through hind limb distention) to obtain good-quality semen. Although there were urates released when the probe was inserted into the cloaca, the region was cleaned and dried before semen collection and there was no semen contamination. The semen was collected from the tip of the ejaculatory sulcus because the movement of the probe provokes the contraction of the vas deferens, leading the semen to the tip of the phallus (Platz et al., 1980). Larsen and Cardeilhac (1994), O’Malley (2005), and Johnston et al. (2014) did not obtain good quality samples in caimans and crocodiles due to the high contamination with urates released by the sedated animals, as kidneys, ureters, testicles, and vas deferens were all stimulated when non-specific electrical stimulation was applied.

Collection by manual massage at the base of the phallus was also effective in spite of the release of urates and feces that occurred when the phallus was exposed. The region was cleaned to prevent semen contamination and the sample was collected straight from the tip of the ejaculatory sulcus. Romero-Solerzano et al. (2010) and Johnston et al. (2014) also obtained samples without contamination from urates using manual massage of the distal portion of the vas deferens and in the direction of the proximal length of the phallus in large crocodiles that were sedated and placed in ventral decubitus. In other reptiles, including snakes (P. guttatus; Fahrig et al. 2007) and lizards (Oligosoma maccanni, O. grande and O. otogense; Molinia et al. 2010), the semen collection was successful and the obtained semen had good quality (92.5% and 70% motility, respectively).

The three collection techniques used in Caiman crocodilus in this study proved to be efficient for semen collection. Animals did not show discomfort or side effects and were released immediately after collection, without the need for any other auxiliary procedures.

Post-mortem collection yielded samples of good quality in terms of cells, but with large concentrations of mucus from the vas deferens. Several dilutions were needed in order to access the spermatozoa. The results reported in this study are similar to those of Larsen and Cardeilhac (1984), who used catheterization of the ejaculatory sulcus and compression of the vas deferens in alligators. Post-mortem spermatic cells have also been obtained from other reptiles (e.g., turtles [Chrysemys picta, Trachemys scripta, Sternotherus odoratus; Gist et al. 2000], through the rupture of the epididymis ductus and the draining of the spermatozoa into the Krebs–Ringer phosphate for 10-15 minutes. In tegus (Tupinambis merianae), Young et al. (2007) obtained spermatozoa through slicing and washing of the vas deferens with 0.9% saline solution. In our study, post-mortem collection was efficient in yielding samples that were adequate for analysis, but the presence of high amounts of mucus is undesirable for semen processing. Given the success and relative ease of semen collection from live animals, there were no obvious benefits to euthanizing the animals, which is also not desirable in terms of species conservation.

Conclusions

The methods of electroejaculation and manual massage of the base of the phallus used in this study for semen collection from Caiman crocodilus were efficient. These techniques may be useful in assisted reproduction and conservation breeding programs or in commercial production of crocodilians for food or leather.
References


Accepted by Andrew Durso