Available online at www.sciencedirect.com
ScienceDirect
Research in Veterinary Science 82 (2007) 93–98
www.elsevier.com/locate/rvsc

Sperm motility parameters to evaluate the seminal quality of *Boa constrictor occidentalis*, a threatened snake species

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Accepted 2 June 2006

Abstract

Semen quality analysis constitutes a powerful tool to evaluate the fertility potential of males in threatened species. The Argentine boa constrictor or lampalagua (*Boa constrictor occidentalis*) is a threatened snake species and has been included in Appendix I of CITES. The objective of this work is to characterize the sperm of *B. c. occidentalis* on the bases of dynamic parameters to improve this species conservation. Dynamic parameters were measured in sperm samples using videomicroscopy and image analysis software. The sperm population showed a high degree of heterogeneity in velocity parameter values and 95% of the cells showed a linear pattern of movement. Studies in other species indicate that the number of motile spermatozoa and their movement speed is directly correlated with fertilization success. This work will help to establish basic parameter values for the evaluation of the reproductive potential of populations of *B. c. occidentalis* and to resolve questions referred to its reproductive strategies.

Keywords: Sperm quality; Dynamic parameters; Snake; Threatened species; *Boa constrictor occidentalis*

1. Introduction

Reproductive physiological studies in rare or threatened species provide solutions to wildlife conservation and contribute with useful data for research on economically important animals (see, for example, Busso et al., 2005). Particularly, semen quality analysis constitutes a powerful tool for the creation of gene banks for species conservation (in captivity and in nature) and to evaluate the fertility potential of males (Goeritz et al., 2003) providing remarkable contributions from the areas of assisted reproduction (Howard et al., 1991). Studies concerning semen characterization and sperm dynamic parameters in reptiles are scarce (see Cuellar et al., 1972; Depeiges and Dacheux, 1985; Hamid and Akbarsha, 1989; Velmurgan, 1992; Gist et al., 2000); in ophidians, even preliminary characterizations are unknown.

The Argentine boa constrictor (*Boa constrictor occidentalis* – Philipi, 1873) is a large (up to 3 m), viviparous, constricting snake (Bertona and Chiaraviglio, 2003), whose populations are declining due to strong illegal hunting and capture pressures for skin trade and as pets (Ávila and Acosta, 1996; Gruss, 1991) and severe modification of its habitat as a result of intense farming (Cardozo et al., 2004). As a consequence, this snake is considered a

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doi:10.1016/j.rvsc.2006.06.001

threatened species (Scrocchi et al., 2000) and has been included in Appendix I of CITES (1997). The mating system of this species has been described as prolonged mate search polygyny with the formation of mating groups from April to August (Bertona and Chiaraviglio, 2003), when the female mates with more than one male. Such mating strategy would imply the existence of high levels of sperm competition between males courting a single female.

Spermatozoa of *B. c. occidentalis* share a similar morphology with the general model described for snakes by Oliver et al. (1996). These cells are filiform, presenting a narrow curved head, with its anterior portion covered by a small conic acrosome (Tourmente et al., 2006). Similarly, they also feature the great elongation of the midpiece characteristic of the ophidian spermatozoa (Jamieson, 1999) given that, in *B. c. occidentalis*, this region is three to four times longer than the head (Tourmente et al., 2006).

Sperm motility, evaluated as the sperm velocity and the percentage of motile spermatozoa, is positively correlated to competitive (Birkhead et al., 1999; Burness et al., 2004; Gage et al., 2004) and non-competitive (Froman et al., 1999) fertilization success in several species. Faster sperm should be more competitive during sperm competition because they could reach the egg more rapidly than slower sperm (Snook, 2005). The objective of this work is to characterize the sperm of *B. c. occidentalis* on the basis of dynamic parameters as a contribution to the comprehension of its reproductive strategies, which would lead to improve the species conservation.

2. Material and methods

2.1. Specimens

Males of Argentine boa constrictor were captured from active mating groups belonging to populations located in the northern area of Córdoba Province (29°55′ S; 64°20′ W) during the mating season. Reproductive condition was confirmed by ultrasound scanning of the testes (Toshiba Sonolayer SSA – 270A, linear 7.5 MHz transducer) according to Bertona and Chiaraviglio (2003). Seven mature males fulfilled the required reproductive condition.

2.2. Semen collection

In order to decide on the optimal technique for semen collection in ophidians with high muscular development, two procedures were compared. Initially, the technique described by Mengden et al. (1980) was tested. This procedure consists on ventro-lateral stroking to achieve a relaxation of the musculature of the animal and later massaging the anal plate, which produces a distention of the cloacal musculature. As a second technique (Fig. 1A), the base of the hemipenises was rubbed with a 1.5 mm diameter plastic probe (which was inserted 5–7 cm into the cloacal aperture) in a back and forward motion to achieve the eversion of the hemipenises and subsequent ejaculation.

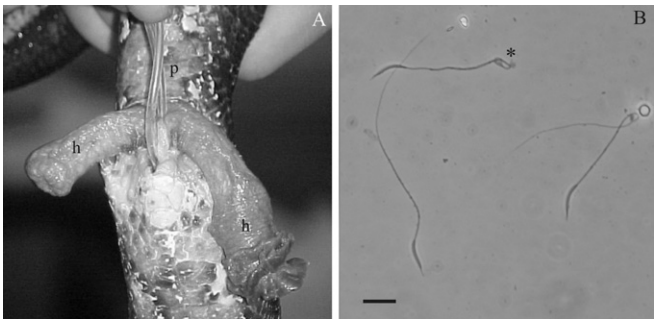

Fig. 1. (A) Photograph detailing the semen collection procedure based on the rubbing of the base of the hemipenises (h) with a plastic probe (p). (B) Phase contrast microscopy image of the spermatozoa of *Boa constrictor occidentalis*. Spermatozoon marked with * has its tail coiled. Scale bar in (B): 20 μm.

Because none of the two afore-mentioned procedures was judged to be traumatizing for the specimens and the fact that abnormal ejaculation had been reported before in anesthetized animals (Dooley and Pineda, 1986; Howard et al., 1986; Pineda and Dooley, 1994), anaesthesia was not used in any of the ejaculation methods. The specimens were restrained by manual immobilization of the head and cloacal region in order to avoid any aggressive reaction to manipulation. The specimens reacted to the technique by relaxing the cloacal muscles, which made the ejaculation possible.

With the purpose of reducing the contamination of the semen samples with feces, the vent was rinsed with sterile physiological solution. Sperm samples were diluted at 50% in phosphate-buffered saline (PBS) 1× and 100 μl aliquots were taken for measurement of dynamic parameters.

All specimens survived to the procedures and none showed any perceptible alterations in its behavior posterior to handling. Specimens were released to the wild approximately two months after semen collection.

2.3. Sperm dynamic parameters

Sperm analysis was carried out under a video-microscopy system consisting of a CCD video camera (Panasonic, MV-5740), a time lapse video recorder (Panasonic, AG-6720), and a monitor (Panasonic, Matsushita Ltd., Osaka, Japan), connected to a Zeiss phase-contrast microscope (Giojalas and Rovasio, 1998; Fabro et al., 2002). All measurements were made at the ambient temperature of the laboratory which, in this case, was 25 °C. Given the lack of group-specific data (with the exceptions of Cuellar et al., 1972; Depeiges and Dacheux, 1985; Hamid and Akbarsha, 1989; Velmurgan, 1992; Gist et al., 2000; for other reptilian groups), we chose standard laboratory conditions for routine sperm physiology studies. Therefore, we diluted the sperm samples in PBS 1x, pH 7.4, to a concentration of 1×10^6 cells/ml. Subsequently, 25 μl aliquots of sperm diluted solution were placed in observation chambers consisting of an acrylic microscopy slide with an excavated circular well. The samples were recorded at 100×