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Seasonal morphological variation of the vas deferens of scorpion mud turtle (Kinosternon scorpioides)

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Abstract: This study aimed to characterize the morphology of the vas deferens of Kinosteron scorpioides by macroscopic and microscopic analysis. Were used 20 adult male jurarás collected at regular intervals during the year and divided into four experimental groups in the rainy and dry seasons, being processed for light microscopy, scanning electron microscopy and transmission electron microscopy. Morphometry for tubular and luminal diameters and epithelial height were also performed. On rainy season, vas deferens presented pseudostratified epithelium with cylindrical cells, spermatozoids and milky fluid in the lumen, with cytoplasmic organelles and lipid vesicles. On dry season, epithelium was pseudostratified with cuboid cells, with cellular debris and no spermatozoids. There was significant variation (p < 0.05) for morphometry of vas deferens, with lower values of tubular and luminal diameters on rainy season, and higher epithelial height on dry season.

Keywords: Kinosternon, Reproduction, Vas deferens, Morphometry, Ultrastructural.

Introduction

Brazil has 35 species of chelonians distributed in its various terrestrial and aquatic ecosystems, of which 28 species are freshwater, two are terrestrial (land turtles), and 5 are marine turtles (SBH, 2005). The family Kinosternidae is composed of semi-aquatic species of small to medium size, being distributed from Canada to South America (Erns & Barbour 1989). It is composed of 22 species subdivided in four genus: Kinosternon, Sternotherus, Staurotypus and Claudius. In the Brazilian Amazon it is possible to found only one species of this family, Kinosternon scorpioides, also known as scorpion mud turtle (Molina & Rocha 1996).

The scorpion mud turtle is preferably an aquatic species, and inhabits both stagnant and flowing water, being also able to develop semi-aquatic behavior (Pritchard & Trebbau 1984). It displays a shell with three evident keels, especially the median, which runs through the shell in the longitudinal

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direction (Vanzolini et al. 1980). It also has a strong jaw and a structure similar to a termed scientifically this species.

*Kinosternon scorpioides* is well distributed in the coast of South America, including Colombia, the Guianas and Trinidad. In Brazil it is found in the states of Pará, Maranhão, north of Goiás, Ceará, Rio Grande do Norte and Pernambuco (Pritchard & Trebbau 1984). In Maranhão its presence is confirmed on the edge of rivers (Pereira 2007), and it is considered an important species, both economically and as a source of protein.

The male reproductive system consists on a pair of oval testes of variable size, between light yellow to golden yellow color, and being fixed by mesorchium and mesocolon; epididymis located along the dorsal part of medial surface of each testis, being very delicate, presented as very convoluted epididymis located along the dorsal part of medial surface of color, and being fixed by mesorchium and mesocolon; testes of variable size, between light yellow to golden yellow color, and being fixed by mesorchium and mesocolon; testes of variable size, between light yellow to golden yellow color, and being fixed by mesorchium and mesocolon; testes of variable size, between light yellow to golden yellow color, and being fixed by mesorchium and mesocolon; testes of variable size, between light yellow to golden yellow color, and being fixed by mesorchium and mesocolon; testes of variable size, between light yellow to golden yellow color, and being fixed by mesorchium and mesocolon; testes of variable size, between light yellow to golden yellow color, and being fixed by mesorchium and mesocolon; 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with cuboid cells, absence of spermatozoa in the lumen and cellular debris.

Scanning electron microscopy of vas deferens by cryofraction technique showed disposition of spermatozoids on tubular epithelium and arrangement of dense connective tissue along to a muscular layer close to the blood vessels. It is believed that these elevations favor storage of sperm in the region (Figure 2).

Transmission electron microscopy on rainy season showed spermatozoids, cytoplasmic organelles (mitochondria), indicating high metabolic activity, and lipid vesicles responsible for nutrition of spermatozoid during storage. On dry season, ultrastructural findings were disorganized and sparse cytoplasm, with endoplasmic reticulum, indicating protein production, and few spermatozoids (Figure 3).

There was significant variation (p<0.05) between seasons for morphometry of vas deferens. The tubular and luminal diameters had lower values on rainy season. However, epithelial height in dry season had higher averages (Table 1).

Discussion

Although in scorpion mud turtle the vas deferens expands in a shape of a small bulb, this feature was not observed for the same species by Carvalho et al. (2010), which does not report the expansion of vas deferens before its insertion on the cloaca. Chaves (2011), however, described the same characteristic, characterizing this expansion in bulb shape on vas deferens of scorpion mud turtle.
By light microscopy, in snakes (Bothrops jararaca and Crotalus durissus), epithelial cells showed microvilli which were not observed in scorpion mud turtle, even though similar secretion was observed by the epithelium (Almeida-Sousa 2005). Similarity was described on close species, such as the crocodile (Guerrero et al. 2004), and birds, particularly in the rooster (Tingari 2001). In the snake Seminatrix pygaea of South Carolina, was observed arrangement of spermatozoids tangent to the epithelium, most being slightly separated from epithelium (Ssever 2004).

Structural morphology was also closed to that observed in snakes, presenting lots of spermatozoa in the lumen of vas deferens in rainy season, indicating copulation period, when it is used as a storage organ; and decrease of spermatozoa in the lumen on dry season, indicating a postmating stage (Rojas et al. 2013). However, in rat snakes, although vas deferens is also the main sperm storage organ, spermatozoa is present in large numbers throughout all the year, except in July (Gang et al. 2011).

Scanning electron morphology in snakes reported vas deferens as being an organ of spermatozoids storage (Almeida-Sousa 2005). In this study of scorpion mud turtle, it is believed that vas deferens is also adapted for storage, due to its structural characteristics such as absence of cilia or stereocilia in the cells. On crocodiles (Caiman crocodilus), non-ciliated cells were also found, indicating storage function of vas deferens in another species of reptiles (Guerrero et al. 2004).

Transmission electron microscopy also presented lipid vesicles in snakes on the rainy season, which are responsible for nutrition of spermatozoa (Rojas 2013). On dry season, the visualization of spermatozoa in the lumen demonstrates that the organ is in reproductive activity. On domestic quails, was reported that vas deferens showed little annual variability, with a significant increase in tubular caliber, intraluminal storage of spermatozoa and occurrence of mitochondria, lysomes, endoplasmic reticulum and variable vesicles in the cytoplasm of principal cells. These ultrastructural features of principal cells seems to be indicative of the occurrence of active processes of endocytosis, and degenerative characteristics were observed at the supranuclear cytoplasm of epididymary P cells on autumn (Orsi et al. 2007). On crocodiles, endoplasmic reticulum was also abundant, indicating protein production, despite of absence of visible secretory material (Guerrero et al. 2004).

By morphometry, the decrease of tubular and luminal diameters, along with increase of epithelial heights on rainy season, are correlated to seasonal variations in synchrony with the spermatogenic and epididymal cycles. In the same sense, was described in snakes (Cerastes viper and Psammophis sibilans), a larger diameter and short epithelial linings during reproductive season as a result of elongation of stored spermatozoids (Sivan et al. 2012, AMER et al. 1978).

Table 1. Mean and standard deviation of morphometry (mm) of tubular and luminal diameters and height of the vas deferens of turtle (Kinosternon scorpioides), captured in São Bento - MA, according with the season. São Luis - MA ~ 2012.

<table>
<thead>
<tr>
<th>Vas deferens</th>
<th>Rainy season</th>
<th>Dry season</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mar/11</td>
<td>jun/11</td>
</tr>
<tr>
<td>Tubular diameter</td>
<td>395.72 ± 60.77 a</td>
<td>298.96 ± 50.50 ab</td>
</tr>
<tr>
<td>Luminal diameter</td>
<td>292.99 ± 59.46 a</td>
<td>243.68 ± 75.68 ab</td>
</tr>
<tr>
<td>Epithelial height</td>
<td>37.24 ± 13.65 a</td>
<td>18.67 ± 5.52 b</td>
</tr>
</tbody>
</table>

Means in different letters in the same line indicate statistical difference ($p<0.05$) for Student-Newman-Keuls (SNK), normality test t Cramer-von Mises W-Sq 0.03979, Pr $> W$-Sq $> 0.2500$. Dec - December. Mar - March. Jun - June. Sep - September

We conclude that vas deferens is the main sperm storage organ on the scorpion mud turtle, presenting a large number of spermatozoids in reproductive season, and morphological findings that represent an adaptation to its function. However, on dry season, the storage was smaller, indicating that organ is in reproductive activity, but production of spermatozoids is reduced in comparison to the rainy season. Further studies on hormonal levels and quality of spermatozoids are suggested, in order to refine the knowledge on the reproductive biology of *Kinosternon scorpioides*.

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