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Histological Study of the Male Internal Genital Tract of Dwarf Caiman (*Paleosuchus palpebrosus* Cuvier, 1807)

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ABSTRACT

Brazil has six species of crocodiles belonging to the subfamily Caimaninae, one of them being *Paleosuchus palpebrosus* (Cuvier, 1807) (dwarf caiman). It is considered one of the smallest crocodylian species, and is sensitive to environmental changes. The anatomical and histological descriptions of the reproductive tract of reptiles are scarce, so it is important to study the morphology of the genital tract of caimans for a better understanding of the reproduction of these species. We performed the histological analysis of the testis, epididymis, and deferent duct of the dwarf caiman *Paleosuchus palpebrosus*. Structures of the genital tract were collected from euthanized animals. Testes, epididymis, and deferent ducts were separated and prepared according to routine histological techniques for morphological characterization. The testis is covered by a capsule of fibromuscular connective tissue. Spermatogonia are observed as spherical cells with round nuclei and loose chromatin, and primary spermatocytes show dark cytoplasm, round nuclei, and pale chromatin. Spermatids are seen as cylindrical or filamentous cells with nuclei that may be highly condensed, ranging from round to oval. The epididymis is observed as a coiled duct lined by a tall cuboidal epithelium with stereocilia; the deferent duct shows ciliated pseudostratified cylindrical epithelium. The adrenal gland is found in the dorsomedial portion of the testis. The histological structure of the reproductive tract of *Paleosuchus palpebrosus* is similar to that of other reptiles' species.

1. Introduction

Brazil has six species of crocodiles belonging to the subfamily Caimaninae: *Caiman latirostris* (Daudin, 1802) (broad-snouted caiman), *Caiman crocodilus* (Linnaeus, 1758) (spectacled caiman), *Caiman yacare* (Daudin, 1802) (swampland alligator), *Paleosuchus palpebrosus* (Cuvier, 1807) (dwarf caiman), *Paleosuchus trigonatus* (Schneider,

1801) (smooth-fronted caiman), and *Melanosuchus niger* (Spix, 1825) (black caiman)^[1]. *Paleosuchus palpebrosus* is considered one of the smallest crocodylian species, reaching between 100 to 150 cm in length^[2]. It is found both in clearwater or blackwater rivers, showing preference for cold water, and it is known to live in burrows whose entries are located below water level. The species is sensitive to environmental changes, and it is difficult to

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be maintained and bred in captivity^[3].

Crocodylian reproduction, both in natural and captivity conditions, has been receiving attention because of animal management. Environmental factors, such as temperature and water level interfere with gonad activity. For captive animals, improving quality and quantity of food is essential in the stabilization of reproductive periods^[4].

Anatomical, morphology and histological descriptions of the male reproductive system of reptiles in general are scarce, but this knowledge is very important to determine the role of these tissues in the production of functional sperm^[5]. Gribbins^[6] provided the first complete histological analysis of spermatogenesis and the germ cell development within a species of the order Crocodylia and relate these observations to the accumulating data that suggest that temperate reptiles retain a temporal germ cell development pattern similar to anamniotes. These authors also state that alligator's primitive reptilian characters and its important phylogenetic placement between dinosaurs and birds makes it ideal for comparative, developmental, and evolutionary studies on the vertebrate reproductive system. Nixon^[5] showed in your research that the general structural feature of the male crocodile excurrent duct system is a reflection of its common ancestry with Archosaurs, as they share closer similarities with those of birds than other clades within the reptilia or mammalia class.

Cabrera^[7], in the histological description of the male genital apparatus of *C. crocodylus*, reported the presence of testicles, epididymis and adrenal gland coated by capsule of dense fibromuscular tissue, the adrenal gland located on the dorsomedial surface of the testes, and the testes with great spermatogenic activity. In reptiles, testes show seminiferous tubules lined by germinative cells associated with Sertoli cells and the ductal system consists of the rete testis, epididymis and deferens duct^[8,9].

The duct system comprises comprising ductus efferent, epididymis and ductus deferens. The epithelium delineating is dominated by non-ciliated and ciliated cells structured into a simple columnar lining of the ductus efferent through to the high pseudostratified columnar epithelium of the epididymis and ductus deferens. The morphology of these ducts suggests their involvement in seminal fluid production and/or its modification, which likely contributes to the nourishment, protection and/or storage of sperm^[5,10].

The reptiles do not possess accessory sexual glands, but the adrenal gland is similar to mammals located suprarenal and shows a close relationship with the gonads^[11], and is found closely attached to the epididymis^[8,9]. The adrenal gland is formed by two portions: a steroidogenic tissue and a chromaffin tissue, and its distribution in turtles and

crocodylians is abundantly intermingled, but the hormones produced are almost the same between the vertebrate classes, the chromaffin tissue produces adrenaline and noradrenaline and the steroidogenic tissue produces most of the steroid hormones present in mammals^[11,12]. The adrenal gland plays a pivotal role in regulates metabolism, maintains normal electrolyte balance, in the response to predictable stressors of animal daily and seasonal life such reproduction, development and migration^[11,12].

Because of the few reports on the morphology of the genital tract of caimans, it is important to study these organs for greater understanding of the reproduction of those species.

Therefore, the objective of this study was to evaluate the histological characteristics of the testes, epididymis, and deferent ducts of the *Paleosuchus palpebrosus*.

2. Material and Methods

The research used two crocodiles of the species *Paleosuchus palpebrosus* kept in captivity in the Laboratory for Teaching and Research in Wild Animals at FAMEV/UFU. After immobilization, the animals were euthanized with administration of 2.5% sodium thiopental (5 ml) followed by intravenous injection of 19.0% potassium chloride (10 ml), according to the recommendations of the Brazilian Guide of Good Practices for Euthanasia in Animals of Federal Council of Veterinary Medicine^[13]. Samples were collected from the portions of the genital tract (testicles, epididymis and vas deferens). The study was authorized by license SISBIO number 13159-1 and protocol CEUA/UFU number 112/2014.

During necropsy of animals, the testes together with the epididymis and part of the vas deferens were quickly separated from the rest of the reproductive tract, placed in plastic bags, and kept in ice at 5°C during the processing. Samples of these structures were collected for histological analysis. Fragments of tissue of about 1 cm³ were fixed in Bouin's solution and submitted to prefixation for approximately 20 minutes. Then the samples were immersed in 10 % buffered formalin for 48 hours. The material through infiltration was dehydrated in increasing concentrations of ethyl alcohol (starting in 70% alcohol and ending in absolute alcohol) and subsequently cleared in Xylol. Subsequently, the samples were infiltrated with liquid paraffin heated between 56°C-60°C and then embedded in paraffin blocks. Finally, 5 µm thick sections were cut from the blocks, placed on glass slides and stained with hematoxylin-eosin. The slides were digitalized on a Scanscope AT slide scanner (Leica Biosystems®) and evaluated by the software Aperio ImageScope®.

3. Results

The testes of *Paleosuchus palpebrosus* are elongated ovoid surrounded by a layer of adipose tissue, are covered by a capsule of dense fibromuscular tissue well vascularised, equivalent to the tunica albuginea (Figure 1A), which also covers the epididymis and the adrenal gland. Trabeculae of connective tissue completely or partially divide the testis in testicular lobules, which include the seminiferous tubules. The seminiferous tubules are lined by the germinative epithelium which comprises spermatogenic line cells. Cells at different phases of division can be observed (Figure 1B). Spermatogonia are found at the base of the seminiferous epithelium and are characterized as round cells with round nuclei and loose chromatin. They undergo mitosis and yield primary spermatocytes, which show dark cytoplasm with evident nucleolus and loose chromatin (Figure 1B). Spermatids are predominant in the seminiferous epithelium and found in groups close to the lumen of the seminiferous tubule. Young spermatids are cylindrical or filamentous, and their nuclei range from round to oval, with more condensed nuclei and pale cytoplasm. In the lumen, basophilic spermatozoa are found (Figure 1B).

Sertoli cells are also identified in the testes. They show large pleomorphic and pale nuclei with one or two prominent nucleoli (Figure 1B). Figure 1C shows some cells in the interstitial compartment, most likely Leydig cells. These cells are polyhedral with acidophilic cytoplasm and few vacuoles, round nucleus with one or two more prominent nucleoli and loose chromatin. A particularity finding in these animals is groups of clonic spermatocytes (Figure 1C) formed by primary spermatocytes in the basal compartment of the seminiferous tubules. Secondary spermatocytes are also found at the base of the germinative epithelium. However, they are difficult to observe as they undergo a quick second meiotic division. Some spermatogonia may also be found in this location.

The ductus efferent and epididymis were not easily definable on gross dissection but extend from the outer lateral margin of the testis. The epididymis (Figure 1A, D) is composed by a thin convoluted duct, lined with tall cuboidal epithelium with stereocilia and portions of pseudostratified cells. In the lumen of the duct, slightly basophilic spermatozoa are found. The epithelium is surrounded by a layer of circularly oriented smooth muscle fibres.

A structure with glandular characteristics is attached to the dorsomedial portion of the testes, surrounded by a large amount of connective tissue (Figure 2 A, B). This gland probably represents the adrenal gland.

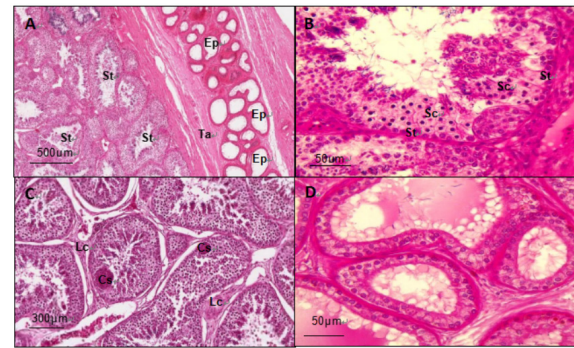


Figure 1. Photomicrograph of the testis and epididymis of *Paleosuchus palpebrosus*. (A) Testis and epididymis; (B, C) Seminiferous tubule; (D) Epididymis. Seminiferous tubule (St); Epididymis (Ep); Tunica albuginea (Ta); Spermatogenic cells (Sc); Sertoli cell (St), Clonic spermatocytes (Cs), Leydig cells (Lc). Amplification of 10 X (Figures A, C) and 40 X (figures B, D). Hematoxylin/eosin colouration. Photos by: Adrielly Lopes.

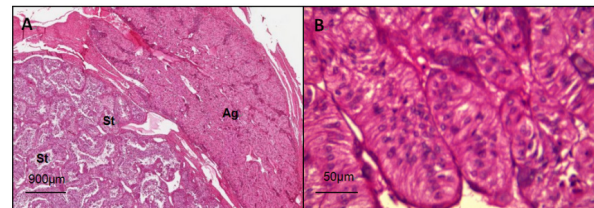


Figure 2. Photomicrograph of the adrenal gland of *Paleosuchus palpebrosus*. Seminiferous tubule (St); Adrenal gland (Ag). Amplification of 10 X (Figure A) and 40 X (figure B). Hematoxylin/eosin colouration. Photos by: Adrielly Lopes.

The epididymis continues in the deferent duct (Figure 3 A, B). It is characterized by cylindrical pseudostratified epithelium with stereocilia and is surrounded by smooth muscle fibres. In the lumen, basophilic spermatozoa are found.

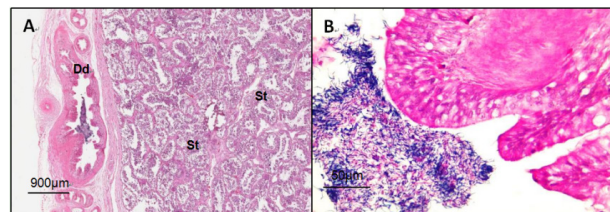


Figure 3. Photomicrograph of the duct deferens of *Paleosuchus palpebrosus*. Seminiferous tubule (St); Deferent duct (Dd). Amplification of 10 X (figure A) and 40 X (figure B). Hematoxylin/eosin colouration. Photos by: Adrielly Lopes.

4. Discussion

The shape of the testes with a tunica albuginea well

vascularised is similar the other alligators, like *Crocodylus porosus* [14]. The capsule of dense fibromuscular tissue surrounding the testis, epididymis and the adrenal gland of *Paleosuchus palpebrosus*, and forms trabeculae in the interstitial compartment, similar to the description by Cabrera [7].

In this study, we have verified the same observation of Gribbins [4,9] who described seminiferous tubules with germinal cells at different phases of division, primary spermatocytes which show dark cytoplasm with evident nucleolus and slightly basophilic spermatozoa in the lumen. In the base of the seminiferous tubules, groups of clonic spermatocytes were observed (Figure B), similar to the structures found in *C. crocodilus* by Cabrera [7], who also characterized secondary spermatocytes as cells with slightly acidophilic cytoplasm and round nuclei. The Leydig cells (Figure C) present in the interstitial compartment, as described by Cabrera [7] in *C. crocodilus*, are similar to those of reptiles, birds and mammals.

Similar histological characteristics of the epididymis (Figure A, D) were reported in other alligators [7,14-17]. Similarly to *Paleosuchus palpebrosus*, in *Crocodylus porosus* the epithelium is formed by non-ciliated and ciliated cells structured into a simple columnar lining of the ductus efferent through to the high pseudostratified columnar epithelium of the epididymis and ductus deferens [5]. Other reptiles, such as sea snakes, also show the same epididymal lining as crocodilians [18]. A similar structure covering the epididymis was found by Guerrero and Saccucci [8,19], which divided this organ in a cranial, middle and caudal portion. In soft-shelled turtle *Pelodiscus sinensis*, the epididymis of the have three distinct regions, cranial, middle and caudal were identified in the based on anatomical characteristics, with epithelium consists of five different cell types: principal, narrow, apical, clear and basal cells [10].

The glandular structure in the dorsomedial portion of the testis that was characterized as an adrenal gland in this study, was similar to adrenal gland identified in other reptiles [7,11,18]. In *C. crocodilus*, similar to that verified in this research, the adrenal gland is closely related to the gonads, with a capsule of fibrous connective tissue and consists of pale cells, corresponding to cortical cells and basophilic cells corresponding to medullary cells [20]. The adrenal gland is formed by two portions: a steroidogenic tissue and a chromaffin tissue, and its distribution in crocodilians is abundantly intermingled, and the organ does not present a clear cortical and medullary division [11,20].

The deferent duct of *Paleosuchus palpebrosus* presented similar characteristics to the descriptions of other caimans [7,8,19], who showed that the deferent duct was covered by the testicular capsule but separated from the

testis by dense connective tissue and its lumen was lined by a cylindrical or columnar pseudostratified epithelium, surrounded by a layer of smooth muscle fibres arranged in a circular direction and an outer layer composed of dense and irregular connective tissue.

5. Conclusions

The structure of the reproductive tract of *Paleosuchus palpebrosus* is histologically similar to that of other caiman species. A peculiarity of the germinal epithelium is groups of clonic spermatocytes in the basal compartment of the seminiferous tubule. The epididymis is very small compared to the size of the testis and the adrenal gland is found in the dorsomedial portion of the testis.

Author Contributions

- Teresinha Inês de Assumpção: Conceptualization, Methodology, Investigation, Resources, Data Curation, Writing, Supervision, Project administration.

- Adrielly Julien Silva Lopes: Methodology, Investigation, Data Curation

- Lilja Fromme: Conceptualization, Methodology, Investigation, Data Curation, Writing

- André Luís Quagliatto Santos: Methodology, Investigation, Resources, Writing

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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