MORPHOLOGICAL OBSERVATIONS ON THE TESTES OF
Physalaemus cuvieri (AMPHIBIA, ANURA)

ESTUDIO MORFOLÓGICO DE LOS TESTÍCULOS DE Physalaemus cuvieri (AMPHIBIA, ANURA)

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SUMMARY: In general, the testes in the anurans are paired ovoid organs, constituted by a mass of seminiferous structures surrounded by a layer of fibrous connective tissue, which holds a germ epithelium with characteristic cellular types. This study describes the testicular histological architecture and anatomy, as well as the organization and morphology of germ cells. Five male samples, from the Botucatu area (São Paulo State, Brazil), of Physalaemus cuvieri (Leptodactylidae) were used. After macroscopic analyses and obtainment of the testicular fragments, the material was submitted to the histological routine to inclusion in paraffin and coloration with haematoxylin/eosin. A rare peculiarity is the presence of numerous pigment-containing cells randomly distributed in the albuginea tunic and testicular interstitium, giving the testis a dark brown coloration. In the germ tissue the spermatogonia I are biggest spermatogenetic cells. With the cellular differentiation and proliferation, succeeded the other cellular types (spermatogonia II, spermatocytes I and II, spermatids I and II, and spermatozoa) with a cystic organization, that is, groups of cells associated with Sertoli cells, forming the spermatogenetic cysts or spermatocysts. The spermatogenetic lineage cells were differentiated and identified according to the cellular and cystic morphology.


INTRODUCTION

In the anurans, the testes are paired ovoid organs constituted by seminiferous tubules convoluted surrounded by the fibrous connective tissue, which constitutes the albuginea tunic. Concerning to the histological architecture of the seminiferous elements, to the amphibians and, in a general sense, the germ epithelium may be arranged in seminiferous locules in the Apoda (Wake, 1969) and Anura (Duellman & Trueb, 1994) or in seminiferous ampoules or testicular lobules the Urodela.

The germ tissue, which constitutes the testicular parenchyma, has different cellular types: spermatogonia in the epithelium boundary; spermatocytes and spermatids in the sequence of the cellular differentiation; and spermatozoa in the lumen or in their adjacencies. In this epithelium, there is a cystic arrangement, that is, groups of sexual cells associated with the Sertoli cells forming spermatogenetic cysts or spermatocysts. Therefore, each one of these units clusters cells in the same stage of differentiation and with a synchronism development, common characteristic in the amphibians (Wake; Lofks, 1974; Rastogi et al., 1988; Oliveira et al., 2002).

There are few researches about the organs and structures that constitute the anuran male reproductive system, specifically in animals of neotropical areas, like in Brazil. Beside that, even if there is not a large number of amphibians in this taxon, several different reproductive strategies will occur (Duellman & Trueb), which seem to be suggestive of morphological and functional variations in the reproductive organs.

Thus, this study tried to analyze the testes morphological arrangement, describing histological and anatomical aspects, as well as some structural characteristics of the germ cells and their cystic arrangement in the anuran Physalaemus cuvieri (Fitzinger, 1826 - Leptodactylidae).
MATERIAL AND METHOD

Five male samples of *Physalaemus cuvieri* were used. The specimens were collected in Botucatu (São Paulo State - Brazil), between June and December, when they were in the reproductive activity period. After captured and transported to the laboratory, the individuals were anesthetized with ether and submitted to morphological studies.

The animals were opened through medium incision from the cloaca as far as anterior limbs, exposing the reproductive organs to macroscopic analyses. After the reduction of the testes in small pieces, they were immediately immersed in Bouin fixative solution during 20 hours, washed, and transferred to 70% ethanol solution. Then, the material was sent to the histological routine to be dehydrated in ethanol, clarified with xylol, and embedded in paraffin. Sections of 6 μm were stained with haematoxylin/eosin for histological analyses. The individuals were appropriately preserved as proof material.

RESULTS

Concerning the gonad macroscopic aspect of *Physalaemus cuvieri*, as to color, shape, and size, we can conspicuous anatomic variations. However, histologically there is not a difference in the germ epithelium arrangement. Covering the testis we can find a thin capsule of connective tissue and smooth muscle, the albuginea tunic. The blood vessels, which exist in this testicular capsule, are mainly destined to the parenchyma, where a germ epithelium is arranged in seminiferous locules. Delimited by a loose connective tissue, these locules form morphological units, that is, the seminiferous locules.

![Figure 1](image_url)

Figure 1. A) Testes anatomic aspect of the *Physalaemus cuvieri* with dark brown pigmentation in the albuginea tunic. Dissected gonad ( ) putting in evidence the internal pigmentation and the units denominated seminiferous locules (27x). B) Histology of the seminiferous locule (H/E, 215x) and C) Generalized representation of the cystic germ epithelium: locular wall (1); interlocular tissue (2) with Leydig cells, blood capillary and pigment-containing cells; primary (3) and secondary (4) spermatogonia; primary (5) and secondary (6) spermatocytes; primary (7) and secondary (8) spermatids; spermatozoon bundles (9) and Sertoli cells (10).
Spermatogonia - three stages of differentiation were distinguished. The primordial spermatogonia or primordial germ cells are the most voluminous cells of the spermatogenetic lineage, whose nuclei are irregular and with a multilobular aspect. They present chromatinic granulations, beside a single, eccentric nucleolus. In general, they are very close to the locular wall, and are associated to the Sertoli cells which still have a follicular aspect and the nucleus is falciform. It is also common that these spermatogonia be isolated or joined in small groups, but this does not characterize germ cysts. From mitotic divisions, the primary (I) and the secondary (II) spermatogonia are originated. The secondary ones are smaller, more irregular, and they were identified due to the nuclear compaction degree. The spermatogonia I are organized as cellular cysts with an irregular aspect and hard delimitation, they are located near the locular wall. The spermatogonia II suffer some alterations, which make the nuclei to become more compacted and give

Figure 2. Detail of the histological arrangement of the seminiferous loculi: 1) spermatogonia I; 2) spermatogonia II; 3) spermatocytes I; 4) spermatocytes II; 5) spermatids I; 6) spermatids II; 7) spermatozoa bundles; 8) spermatozoa in the locular lume. (H/E, A and B - 430x; C and D - 850x).

The different cellular types were differentiated and identified according to the morphology and cystic arrangement (Fig. 2), also represented schematically (Fig. 1c).

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elements of the gonads. In Physalaemus cuvieri, the interlocular tissue is relatively scarce, presenting a pigmentation in this tissue and also in the albuginea tunic, giving the testis a dark brown coloration. Between the seminiferous units, they have a inter-locular tissue composed by Leydig interstitial cells, fibroblasts, pigment-containing cells, blood vessels, and some efferent ductules (Fig. 1).
them typical shapes. The Sertoli cells, when associated with the spermatogonia II, present a nucleus with more elongated shape, still semilunar or falciform.

Spermatocytes – they are the result from the spermatogonia II differentiation, the spermatocytes I are cells just smaller than the spermatogonia I. In its nucleus, the chromatin is slightly condensed. The spermatocytes II are haploid cells and quite smaller than the previous, and they are originated in the first meiotic division. Generally, the spermatocytes are observed in different phases of the first meiotic division, presenting different degrees of compactation of the nuclear material. In this stage, the Sertoli cells are more voluminous, the nuclei are less condensed and present the tendency to assume intermediate shape, between elongated and ovoid.

Spermatids – when the spermatocytes II pass through the second meiotic division, they form the spermatids. These, in the primary stage or as round spermatids I, are differentiated from the cysts of the spermatocytes II when some cells show themselves slightly elongated. The spermatids II or elongated are cells with compacted and elongated nuclei, whose cystic arrangement is altered to be organized in bundles sustained by the Sertoli cells. In this moment, the nucleus of the Sertoli cells finds itself as oval or rounded, with chromatin loosely distributed, besides the evident and central nucleolus.

Spermatozoa – they are characterized by an extraordinary nuclear compactation and a cytoplasmic reduction. The spermatozoon head, represented mainly by the nucleus, is slender; and, in general, it turns to the nucleus of the Sertoli cell, in opposition, the tail has a filamentous appearance and is slightly stain, and, generally, turns to the luminar area of the seminiferous locule. The spermatozoa in development are arranged in bundles very well organized, due to the association with the Sertoli cells. When they reach maturity, they are released from the bundle and reach the locular lumen and the efferent ductule.

**DISCUSSION**

The unusual dark brown pigmentation of the testes of *Physalaemus cuvieri* is a peculiarity, which occur due to a presence of numerous pigmented cells (melano-macrophages or typical melanocytes?) randomly distributed in the testicular capsule and interstitium. This unusual characteristic, a testicular pigmentation, rarely is observed in others species, as described in *Physalaemus fuscomaculatus* (Aoki et al., 1969) and *Bombina bombina* (Gollmann et al., 1993). In the anuran *Xenopus laevis*, and other lower vertebrates, the pigment cells can be found in the different organs, constituting an extracutaneous pigmentary system of unknown function (Zuasti et al., 1998).

Concerning the general histological architecture observed in *Physalaemus cuvieri*, it was verified a great similarity to the descriptions of the other species of same family, like *Physalaemus fuscomaculatus* (Aoki et al.) and *Caudiverbera caudiverbera* (Hermosilla et al., 1983); and also with species of the Hylidae family – *Hyla ranki* (Taboga & Dolder, 1991), *Hyla japonica* (Lee & Kwon, 1992); *Hyla pulchella andina* (Montero & Pisanó, 1992); and *Scinax fuscoverius* (Oliveira & Vicentini, 1998; Oliveira et al.). In the species that show a continuous spermatogenesis for all the reproductive period, different cellular types may be identified in only one seminiferous locule, that is, all the cells of the germ lineage occupy the same locule and differentiate themselves simultaneously.

To Leptodactylids, *Telmatobius laticeps* and *Telmatobius pisanai* (Montero & Pisanó, 1990), the histological arrangement is similar, however a potentially continuous cycle occurs, considering that there is a well-defined phase of the intense spermatogenetic activity and other of rest, as it was also described to the hylidae: *Hyla pulchella andina* (Montero & Pisanó, 1992). In anurans like *Nectophrynoides occidentalis* (Zuber-Vogeli & Xavier, 1966), a temperate area bufonidae, the cycle is discontinuous, it influence even more in the gonadal activity and consequently in the dynamic of spermatogenesis. For the neotropical area species, occurs preferentially a continuous reproduction, in which all the cellular types are present for whole year.

With the cystic arrangement, the spermatogenetic lineage cells together with the Sertoli cells form delimited groups by a membranous capsule, which is denominated germ cyst or spermatocyst. We stress the fact that independently of the reproductive cycle type of the species, the cystic arrangement of the germ cells is confirmed as an important characteristic of the anuran amphibians, as well as to other amniotes vertebrates (Lofts; Grier et al., 1980; Grier, 1992). In a similar way to what was obtained for *Bufo woodhouse* (Atherton, 1974), *Caudiverbera caudiverbera* (Hermosilla et al.) and *Scinax fuscoverius* (Oliveira & Vicentini), we also do not observe any specific area of the testis where predominate certain cellular kinds. It is corroborated by the apparently random distribution of the spermatocytes in the seminiferous structures.

Concerning the spermatogenetic lineage cells, the primordial spermatogonia are the biggest cells and rest on the adjacencies of the basal lamina of the seminiferous structures, associated with the follicular cells (Lofts). As to the
spermatogonial types, similar observations to the *Bufo arenarum* (Cavicchia & Moviglia, 1983), *Hyla ranki* (Taboga & Dolder) and to the *Scinax fuscovarius* (Oliveira et al.) were also verified in the species of this research.

The spermatocytes generally are observed in the prophase of the first meiotic division, with different levels of chromatinic and chromosomal condensation. The spermatocytes I are commonly bigger than the spermatogonia II (Lofts; Rastogi et al. and Oliveira et al.). With the spermatocytes I division, smaller cells are originated, the spermatocytes II, which present half the diameter of the origin cells, as we observed in the *Physalaemus cuvieri*.

In *Caudiverbera caudiverbera* (Hermosilla et al.), *Odontophrynus cultripes* (Bão et al., 1991), *Scinax fuscovarius* (Oliveira et al.) and in our analysis, the spermatids I, in a first stage, show a spherical nucleus and thin granulation, after the nuclei become oval and the granular chromatin is distributed homogeneously. The following stages are characterized by a cellular and nuclear elongation, which occur simultaneously with the chromatinic condensation, culminating with the spermatids II formation.

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RESUMEN: Generalmente, los testículos de anuros constituyen órganos ovoides, pares, formados por masa de estructuras seminíferas, envueltos en una capa de tejido conjuntivo fibroso, que contiene un epitelio germinativo provisto de tipos celulares característicos. En este trabajo se describe la anatomía y la arquitectura histológica de los testículos, así como también la organización y morfología de las células germinativas. Se utilizaron cinco ejemplares machos de la especie *Physalaemus cuvieri* (Leptodactylidae), provenientes de la zona de Botucatu (Sao Paulo, Brasil). Tras el análisis macroscópico y la obtención de los fragmentos testiculares, el material se sometió a las técnicas histológicas rutinarias para su inclusión en parafina y coloración con hematoxilina/eosina. Se constató como rara peculiaridad la presencia de numerosas células con pigmento distribuidas de forma aleatoria en la túnica albugínea e intersticio testicular, las cuales otorgan al testículo una coloración marrón oscura. En el tejido germinativo las espermatogonias I son las mayores células espermatogénicas. En la secuencia de la diferenciación y proliferación celulares, se siguen los demás tipos de células (espermatogonias II, espermatocitos I y II, espermatídias I y II, y espermatocistos) que presentan una organización cística, es decir, grupos de células se asocian a las células de Sertoli, formando los cistos espermatogénicos o espermatocistos. Se diferenciaron e identificaron las células del linaje espermatogénético, según la morfología de las células y del propio cisto.


