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Spermatogenesis and Histology of the Testes of the Caecilian, *Chthonerpeton indistinctum*

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ABSTRACT. — **Macroscopically, the testes of *Chthonerpeton indistinctum* consist of a series of oval lobes. The number and size of lobes is variable in *Chthonerpeton indistinctum*. Histologically, the testes are divided into structural units, locules separated by septa of connective tissue. Inside of each locule spermatogenesis takes place in cysts of germinal cells that divide synchronically. Spermiogenesis is divided chronologically into four stages of development based on the shape of the nuclei of the spermatids and their arrangement in the cyst: (1) round, (2) kidney shaped, (3) attenuate, and (4) bouquet arrangement.**

The caecilians are a relatively small group (five families) of legless, predominantly burrowing amphibians, represented in Uruguay only by *Chthonerpeton indistinctum* (Reinhardt & Lutken, 1862). All caecilians have internal fertilization and all members of the Typhlonectidae and Scolecomorphidae and some of the Caeciliidae are viviparous, although the reproductive biology of these species is poorly known. In fact, published biological literature (other than taxonomy) for this species is limited to Lieberman (1939) and Barrio (1969) (reproduction), Barrio et al. (1971) and Wake and Case (1975) (cytogenetics), Carlisky et al. (1969) (excretion), Aguiar (1979) and de Sa (1984) (digestive tract), De Carlo (1980) (respiratory system), Engelke and De Paula (1979) (blood) and Welsch et al. (1977) (oviducts).

This is the first of a series of papers on the morphological and histological aspects of the reproductive biology of *Chthonerpeton indistinctum*.

MATERIALS AND METHODS

Specimens were collected in April and May 1983, from beneath mats of aquatic vegetation, particularly *Eichornia crassipes*, deposited by an unusually severe flood along the shore of the Río de la Plata estuary in the Departamento de Montevidéo (Playas Pocitos, Malvin, Carrasco and Pajas Blancas) and Departamento de San Jose (Playa Pascual), Uruguay. The location of the caecilians prior to the flooding is unknown. Because of these special circumstances that enabled the collection of these specimens, we cannot assign them to a specific period in the reproductive cycle of *Chthonerpeton indistinctum*. Material used in this study was preserved in 10% formalin in the amphibian collection of the Departamento de Zoología-Vertebrados at the Facultád de Humanidades y Ciencias, Universidád Mayor de la Republica Orientál del Uruguay, Montevidéo, under the lot number ZVC-B 2027.

The histological description is based on testes from three males; no important variation was found among these males. Tissue was imbedded in paraffin and sectioned at 5 μm, and stained with haematoxylin-eosin and PAS-haematoxylin as described by Ganter and Jolles (1970). The size of the nuclei in the germinal cells was measured to the nearest 1.0 μm with an ocular micrometer. Fifty nuclei of each cell type were measured. Silver-stained smears of spermatozoa (technique of Elder and Hsu, 1981) also

were made in order to observe sperm morphology and to measure the spermatozoan heads. The slides were photographed using a Vanox model microscope.

Results

The testes of *Chthonerpeton indistinctum* are organized in a fashion typical for Gymnophiona (Spengel, 1876; Wake, 1968, 1977). They are paired, longitudinally oriented organs that are composed of a series of oval, granular, white lobes; these lobes are aligned serially with the long axis of the body and connected to each other by a single collector duct. Transverse ducts extend from the longitudinal duct to the mesonephros. Each lobe is surrounded by a connective tissue capsule (albuginea) which is covered externally by squamous peritoneal epithelium. Considerable variation is present in the number and size of these lobes, and differences also exist between right and left testes in the same individual.

Histologically, each testicular lobe is divided into locules (Seshachar, 1936) separated by septa of connective tissue; these septa are thickest among the central locules. These interlocular septa consist of intercellular substance, cells, fibers and vessels of the vascular system of the testis (Fig. 1A). In the central septa, spermatozoa were present in the lumina of the interlocular ducts; the walls of these ducts are formed by simple cuboidal epithelium with central nuclei.

Spermatogenesis takes place within the lumen of each locule in cellular clutches called cysts (Lofts' cell nests); somatic Sertoli cells form the cyst walls. All germinal cells in a given cyst are in the same stage of development, but each locule contains cysts in different stages of development.

Spermatogonia.—These cells exhibit the largest nuclei of the germinal line (N = 50; range 8.00–16.00 μm; $\bar{x}$ = 10.6 $\pm$ 1.68 μm SD). Generally, individual cells or cysts (composed of few cells) are located in the periphery of the locules where they are recognized easily. Spermatogonia have a high ratio of nucleus/cytoplasm content and also have spherical, basophilic nuclei with granular chromatin. The small amount of cytoplasm is homogeneous and lightly acidophilic (Fig. 1B).

Spermatocytes.—Variable numbers of spermatocytes occur in the cysts. The nuclei of the spermatocytes are round and smaller than those of the spermatogonia (7.00–10.00 μm, $\bar{x}$ = 8.00 $\pm$ 0.9260 μm). The nucleus accepts basophilic stains and the chromatin is filamentous, frequently forming a network. Spermatocytes are packed closely in the cyst and it is impossible to establish their cellular limits, even though a thin layer of vacuolated cytoplasm with acidophilic characteristics fills the internuclear spaces in the cyst (Fig. 1C).

Spermatids.—Spermatids have the smallest nuclei (4.00–6.00 μm, $\bar{x}$ = 5.06 $\pm$ 0.549 μm), and condensed chromatin. The amount of cytoplasm, if present, is minute. Based on changes in both nuclear shape and cellular arrangement, four stages of maturation of spermatids were identified: (1) round (Fig. 1D); (2) kidney shaped (Fig. 1E); (3) attenuate (Fig. 1F); and (4) mature spermatids in a bouquet arrangement (Fig. 1G). During stages 1 and 2, the spermatids are contained within a closed cyst, while in stages 3 and 4, the cyst wall has broken down.

Spermatozoa.—Mature spermatozoa are imbedded in the cytoplasm of the Sertoli cells or are free among the germinal cysts, in the locular lumen. Mature sperm have a relatively large, cylindrical head (length, 15.00–19.00 μm, $\bar{x}$ = 17.34 $\pm$ 0.847 μm), and are highly and homogeneously basophilic. In smears of free sperm stained with silver-nitrate, the flagellum is approximately three times the length of the head (Fig. 1H).

Somatic (Sertoli) cells form the cyst walls. These cells have a triangular-shaped nucleus that is less basophilic

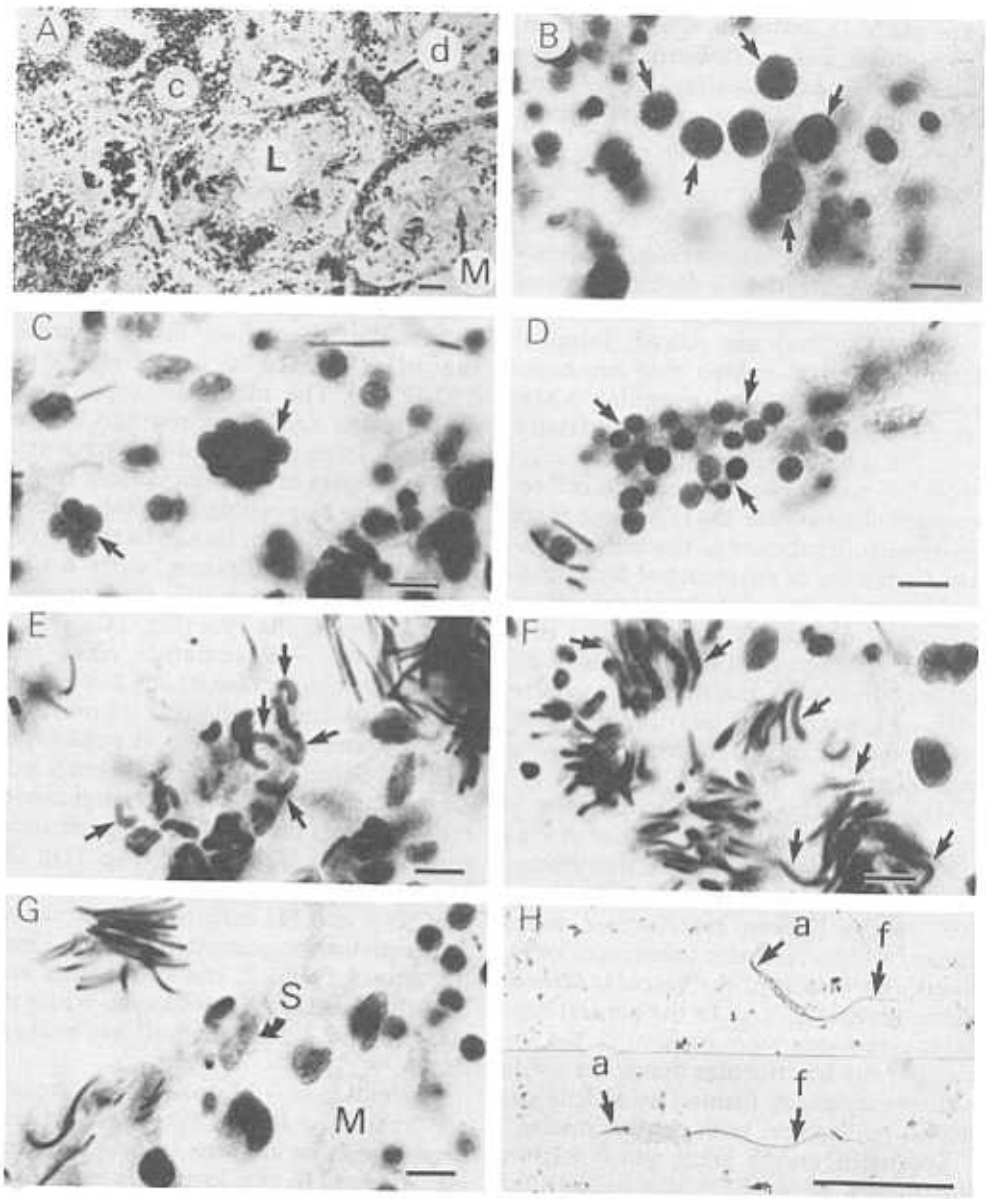
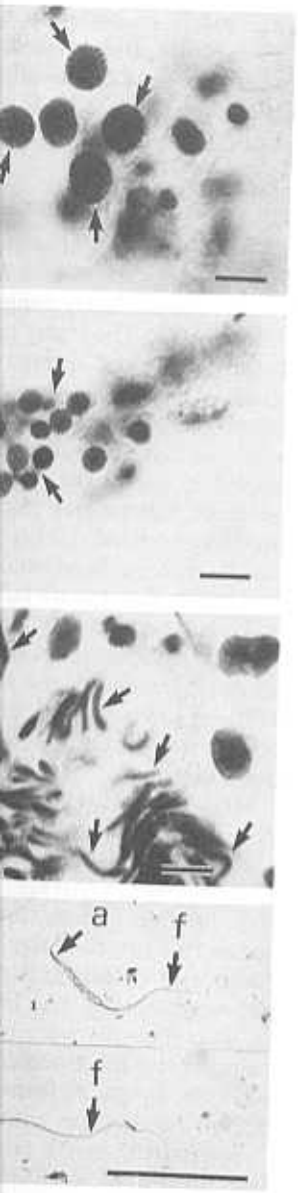


FIG. 1. Spermatogenesis in *Chthonerpeton indistinctum*. A. Topographic view of the testes, L = Locule, c = connective tissue, d = interlocular duct, M = matrix. (Bar = 50 μm, stain = haematoxylin-eosin.) B. Spermatogonia. C. Spermatocytes. D. Round spermatids. E. Kidney-shaped spermatids. F. Attenuate spermatids. G. S = Sertoli cell, M = matrix. (B–G, Bar = 10 μm, stain = haematoxylin-eosin.) H. Mature spermatozoan, a = acrosome, f = flagellum. (Bar = 10 μm, silver-stained smears.)

than those of the germinal cells. Also, Sertoli cells have finely granular chromatin with a large nucleolus that stains deeply. We could not establish the borders of the Sertoli cells precisely, but the cytoplasm itself is characterized by low acidophily and a granular appearance. Among the cysts there is a vacuolate and refringent matrix that stains slightly with eosin.



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Discussion

The macroscopic morphology of the testes of *Chthonerpeton indistinctum* is similar in segmentation and coloration to others described for the order Gymnophiona. The gross morphology of the testes of several species of caecilians was described by Wake (1968) with regard to the number and size of testes lobes, as well as the position of the transverse ducts. In *Chthonerpeton viviparum*, the anterior lobe is the largest (Wake, 1968). Specimens of *C. indistinctum* that we examined exhibited great variation in the number and sizes of the lobes. Also, there are differences between the testes on the two sides of the same individual. Larger lobes possess more transverse ducts than smaller lobes; this agrees with Wake's (1968) hypothesis of an evolutionary trend toward fusion of testicular lobes.

In *Chthonerpeton indistinctum*, spermatogonia are the largest cells of the germinal line. They were found in the locular lumen, most frequently against the locular wall, individually or in cysts formed by few cells. Seshachar (1936) found that the nuclei of primary spermatogonia of *Ichthyophis glutinosus* change from a spherical condition to a polymorphic condition when the cell is in a period of high metabolic activity; reacquisition of the spherical condition by the nucleus denotes the secondary spermatogonia. We did not observe polymorphic nuclei in *C. indistinctum* spermatogonia. This lack could be due either to the fact that spermatogonia in this species do not exhibit this change in nuclear morphology or that the high metabolic stage of the primary spermatogonia is very short and simply was not observed.

Seshachar (1943) divided the development of spermatozoa of *Ichthyophis glutinosus* into five parts, based on changes in the following cell structures: centrioles, acrosome, nucleus, Golgi bodies and mitochondria. The four stages of spermiogenesis identified in this paper accentuate the morphological changes that the nucleus of a spermatid undergoes through the maturation process. In *C. indistinctum*, each spermatid initially has a round and condensed nucleus; these cells are identified as early spermatids. As spermiogenesis progresses, these early spermatids begin to elongate and acquire a kidney-shaped nucleus. Presumably, this shape results because the cells are completely enclosed in a cyst. Later, the nucleus becomes even more elongate and exhibits an attenuate shape. This change to an attenuate shape is influenced by the breaking of the cyst wall. Finally, spermatids with attenuate nuclei are considered mature when they are arranged in a bouquet pattern in which their anterior ends are imbedded in the cytoplasm of Sertoli cells. During this period, the nucleus has acquired the mature spermatozoan morphology with head, acrosome and tail. Spermatozoa were observed free or in clusters in the locular lumina. Our study in *Chthonerpeton indistinctum* showed the presence of an intralocular matrix. This intralocular matrix has been observed in several other species (Wake, 1968).

Chthonerpeton indistinctum retains the typical anamniote pattern, in that spermatogenesis occurs in cysts contained in locules. However, the developmental pattern of spermatogenesis is quite variable among amphibians. In some urodeles, germinal cells develop caudo-cranially within the testes. As a result of this, cells at a particular stage of development are located together and different stages are found sequentially in the testes. This sequential pattern is not observed in *Chthonerpeton indistinctum;* instead, cysts in different stages of development are found in the same locule. Wake (1968:310) reported that this condition—the presence of intralocular cysts in different stages of maturation—is a characteristic unique to caecilians. However, the presence of cysts in different developmental stages within the same locule was reported for *Bufo* (Burgos and Mancini, 1947) and observed

(by both authors) in several other anurans (e.g., *Leptodactylus ocellatus, Hyla pulchella, Physalaemus gracilis*).

The Sertoli cells identified for *C. indistinctum* exhibit characteristics similar to those described for *Bufo* (Burgos and Mancini, 1947).

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