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Histology of the Ovaries and Fat Bodies of 
**Chthonerpeton indistinctum**

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**Abstract.** Histological analysis revealed three different stages in the development of ovarian follicles of *Chthonerpeton indistinctum*. These stages are identified by (1) location of the oocyte in the ovary wall, (2) oocyte morphology and staining characteristics, (3) development of the zona pellucida, and (4) organization of the follicular layer. Atretic follicles and corpora lutea also were found. Fat bodies are composed of well-developed adipose tissue.

The caecilian family Typhlonectidae includes 19 aquatic, viviparous species. Aside from Taylor’s monograph on Gymnophiona (1968) and the work done on *Typhlonectes compressicauda* by Exbrayat (1983), Exbrayat and Collenot (1983), Exbrayat et al. (1981, 1982) and Delsol et al. (1981), little information is available on the reproductive biology of this family. Lieberman (1939) and Barrio (1969) described some aspects of the reproductive behavior of *Chthonerpeton indistinctum* (Reinhardt and Lutken, 1862). Histological aspects of the oviducts of this species were studied by Welsch et al. (1977), and de Sá and Berois (1986) studied the spermatogenesis. Ovarian morphology and histology were described by Wake (1968, 1977) for several species of caecilians, but histological data for *Chthonerpeton* were not included. Classification of ovarian eggs in caecilians has been based on egg sizes (Wake, 1977; Exbrayat and Collenot, 1983).

Review papers dealing with the ovary of nonmammalian vertebrates, such as those in the volume by Jones (1978) and Wallace (1983), base their presentations on the amphibian ovary from work done on anurans or urodeles only. In this paper, we present a histological analysis of the ovary and fat bodies of *C. indistinctum*. It was found that follicular development may be staged according to histological characteristics and preliminary information on atretic follicles is reported.

**Materials and Methods**

Specimens were collected in April and May 1983, from beneath mats of aquatic vegetation (particularly *Eichornia crassipes*) deposited by a flood along the shore of the Río de la Plata estuary in the Departamento de Montevideo (Playas Pocitos, Malvin, Carrasco and Pajas Blancas) and Departamento de San José (Playa Pascual), Uruguay. The origin of these specimens prior to the flooding is unknown. The specimens used in this study were preserved in 10% formalin. Part of the collection (lot number ZVC-B 2027) was deposited in the amphibian collection of the Departamento de Zoología-Vertebrados, Facultad de Humanidades y Ciencias, Universidad Mayor de la República Oriental del Uruguay, Montevideo, and the rest are maintained in the private collection of one of the authors (RDS).

For histological description, ovary samples were double embedded in celloidin-paraffin (Ganter and Jolles, 1970), sectioned at 5 µm and stained with PAS-Hematoylin (red) and alochrom (blue) (Lillie, 1954). The fat bodies were embedded and stained as described for the ovaries. Sections were photographed using a Vanox model microscope.

**Results**

The ovaries of *Chthonerpeton indistinctum* are elongate, sac-like, paired organs, lateral
to the kidneys, and connected to the dorsal wall of the body by the mesovarium. Macroscopically, two kinds of eggs can be distinguished—small, unpigmented eggs and larger, yellowish eggs—dispersed along the length of the ovary (Fig. 1A).

Histologically, the ovaries have an unpigmented wall covered externally by a squamous peritoneal epithelium that contains elongate nuclei with fine chromatin. Under this peritoneal layer, the epithelium of the ovarian wall is simple (with cubic or cylindric cells) in some regions, whereas in others it is stratified. The nuclei of the epithelial cells are homogeneously basophilic.

The germinal cells are scattered among the epithelial cells and are large and round, with weakly staining cytoplasm and some PAS-staining granulations. The nucleus of each germinal cell is round and central and stains light blue with alocrom. Chromatin is homogeneously dispersed, and one or more nucleoli are present. Oogonia are the smallest germinal cells and contain only one nucleolus. Oocytes are larger cells with one or more nucleoli, in association with epithelial cells, the oocytes form the primary follicles (Fig. 1B). The epithelium is delimited by a thin PAS-positive, basal membrane, under which is a fibrous connective tissue with an extensive capillary network.

_Ovarian Follicle._—Oogenesis takes place in follicles that are formed by a central cell, the developing oocyte, in association with the surrounding somatic follicular cells. Follicles are differentiated into primary, secondary, and tertiary forms according to their stages of development as determined by the following criteria: 1) location in the ovary wall; 2) oocyte morphology and staining characteristics; 3) development of the zona pellucida; and 4) organization of the follicular layer.

_Primary Follicle._—Primary follicles are located in the superficial ovarian epithelium. Each follicle is formed by an oocyte with a central nucleus and several nucleoli, although one nucleolus is larger than the rest. A few epithelial cells are flattened against and around the surface of the oocyte. The cytoplasm of the oocyte does not exhibit any major differences from oogonial cytoplasm (Fig. 1B).

_secondary Follicle._—These follicles are located deeper in the ovary wall than primary follicles. Secondary follicles either deform the subepithelial basal membrane or are surrounded almost completely by the connective tissue underlying the basal membrane (Fig. 1C).

The size of secondary follicles is variable with oocyte development. The oocyte cytoplasm at this stage is filled with fine yolk granulations that stain slightly with PAS and blue with alocrom. The oocyte nucleus is eccentric, oval, weakly staining, and a large number of nucleoli are present. The chromatin exhibits fine granulations that may form a network. Secondary oocytes are surrounded by a larger number of follicular cells than are primary follicles, although the follicular layer is still not continuous around the oocyte. At this stage, a thin layer of PAS-positive material begins to accumulate between the oocyte and the follicular cells (Fig. 1D).

_Tertiary Follicle._—Tertiary follicles are the largest, located deepest in the ovary, and are surrounded completely by connective tissue. The cytoplasm of the tertiary oocyte is filled with two types of yolk granulations. The fine granulations described in the secondary follicles now are observed to be peripheral, lying immediately adjacent to the cell membrane of the tertiary follicles. The second kind of yolk granules is larger and deeply PAS-positive. These characteristics and the larger size of the oocyte make this stage of development easily identifiable (Fig. 1E).

The oocyte nucleus of the tertiary follicles is flat, completely eccentric, and compressed against the oocyte wall. It contains homogeneous chromatin and several nucleoli. The PAS-positive material that was first observed between the oocyte and the follicular cells in the previous stage now forms a smooth, continuous layer—the zona pellucida—surrounding the oocyte. The follicular cell layer also completely surrounds the oocyte (Fig. 1F). Follicular cells are tall and have a central, oval nucleus with granular chromatin and a slightly basophilic cytoplasm. The connective
FIG. 1. Oogenesis in Chthomerpeton indistinctum. A. Topographic view of the ovary with oocytes in different stages of development. (Bar = 100 μm, stain = PAS-Hematoxylin). B. Ovary wall, e = epithelial cells, m = basal membrane, f = Primary follicle, g = oogonium, fc = follicular cell. (Bar = 20 μm, stain = PAS-Hem.). C. Ovary wall, g = oogonium, f = Primary follicle, f’ = Secondary follicle, v = vessel. (Bar = 20 μm, stain = Aloechrom). D. f’ = Secondary follicle, p = zona pellucida, fc = follicular cell, n = nucleus; note the large number of nucleoli. (Bar = 50 μm, stain = Aloechrom). E. f’ = Tertiary follicle, n = nucleus. (Bar = 50 μm, stain = PAS-Hem.). F. fc = follicular cell, p = zona pellucida, y = yolk (two types of yolk) (Bar = 10 μm, stain = PAS-Hem.). G. Atretic follicle, cy = cytoplasm with clustered yolk, fc = follicular cell, v = vessel, arrows showing lymphocytes. (Bar = 50 μm, stain = PAS-Hem.). H. Corpus Luteum, st = stroma, p = zona pellucida, arrows showing follicular cells in the stroma. (Bar = 20 μm, stain = PAS-Hem.).
tissue immediately adjacent to the follicular cell layer forms a theca surrounding the follicles. The results of the alchro-mowing technique show the theca to be well-vascularized.

Atypical Follicle.—Several follicles similar in size and general aspect to tertiary follicles also are present in the deeper layer of the ovary. However, these atypical follicles exhibit the following differences from tertiary follicles: 1) the zona pellucida is absent; 2) the large, PAS-positive yolk granulations in the oocyte cytoplasm are aggregated in clusters; 3) a complete follicular layer is lacking, and 4) the surrounding connective tissue also is disorganized and is highly vascularized. In addition, small and deeply basophilic cells, identified as lymphocytes, are present in the oocytes. Each atypical follicle lacks a nucleus (Fig. 1G).

Non-yolked Structures.—Cellular clusters formed by follicular cells that surrounded an internal stroma that totally lacked PAS-positive yolk granulations also were observed. Follicular cells also may occur in the stroma. These structures are connected to the superficial epithelium of the ovary wall, either by a relatively narrow pedicel or a large zone of contact. Connective tissue and irrigation vessels surround these structures externally. Between the peripheral cells and the central stroma there is evidence of PAS-positive material (Fig. 1H).

Fat Bodies.—The fat bodies of Chthonerpeton indistinctum are lobate organs present in both sexes. The number of lobes is variable and each laminar lobe partially overlaps the lobe immediately posterior. Fat bodies extend from the liver to the cloaca along the sides of the coelom and are attached to the mid-dorsal wall by a mesentery that is fused with the mesovarium. In life, they are bright orange-red, but become pale yellow after fixation in formaldehyde.

The fat bodies are composed of well-developed adipose tissue. Each fat cell is polygonal and possesses a large vacuole that appears empty after embedding and staining. An extremely thin, PAS-positive, refringent layer of cytoplasm shows the cellular borders. Each cell has a flat nucleus that is compressed against the cell membrane; the nucleus may appear to be triangular-shaped when compressed against the angles of the cells. Irrigation vessels occur at the intercellular angles.

DISCUSSION

External Morphology.—The morphology of the ovary and its peritoneal relationships in Chthonerpeton indistinctum coincides with Wake’s (1968) observations for other caecilians. Because our specimens were collected at only one time during the year, we observed only individual variation in ovary size and not the annual variation reported for other species. Owing to the circumstances of the collection, we also cannot assign our material to a particular stage in the annual reproductive cycle of the species.

Histology of the Ovary.—Histologically, the ovaries of Chthonerpeton indistinctum exhibit an irregular distribution of the mature oocytes similar to that reported by Wake (1968) for Rhinatremia bicolor and Caecilia tentaculata. Unfortunately, there is no information available for other species of the genus Chthonerpeton.

In Chthonerpeton indistinctum the ovary wall is formed by the ovarian epithelium, here called the germinal epithelium, and is covered externally by peritoneum. The early primary follicles of C. indistinctum lie in the germinal epithelium and the oocyte is surrounded by a few epithelial cells that differ only by their flatness against the oocyte surface. Presumably, the follicular cells of C. indistinctum are derived from epithelial cells that flattened against the primary oocyte and become cubical in later stages of development.

Oogenesis.—Amphibian oocytes are telolecithal. The most evident changes during their maturation occur at the cytoplasmic level at which reserve material accumulates for future embryonic development. Before maturation begins, the nucleus is arrested in first meiotic division until fertilization occurs.

Wake (1977) described previtellogenic and vitellogenic eggs. Macroscopically, these two kinds of eggs can be identified in Chthonerpeton indistinctum, but histologically, three developmental stages could be distinguished based on (1) the position of
the egg in the ovary wall; (2) oocyte morphology and stain characteristics; (3) development of the zona pellucida; and (4) organization of the follicular cells. The primary and secondary follicles of our classification correspond to the previtellogenic eggs of Wake’s classification and the tertiary follicles correspond to vitellogenic eggs.

During development, yolk of two different sizes accumulates in the cytoplasm of the oocyte. One is synthesized early (allochrom-positive) and is clearly seen in primary and secondary follicles. The second kind of yolk is formed by larger granules, synthesized later (allochrom-negative), and in tertiary follicles obscures the early yolk granules. The vitellogenic follicles of Wake’s classification have this larger type of yolk granules. The two yolk types are easily differentiated by the sizes of granules and stain affinities. Wallace (1983) indicated that a large lipoglycoprophosphoprotein (vitellenin) is synthesized in the liver and is the precursor of yolk proteins (lipovitellin and phosphitin) found in the oocytes of nonmammalian vertebrates. Yolk platelets increase in size by either the continual addition of yolk materials (Wallace, 1983) or by the fusion of platelets with one another (Karasaki, 1963). There is no information about the development of caecilian yolk. If the composition of the two kinds of yolk described here in Chthonerpeton indistinctum could be determined, we might identify whether the two types result from different stages of yolk platelets or from different synthetic processes.

Primary and secondary follicles also differ from tertiary follicles in the position of their nuclei, which become progressively more eccentric during maturation from the increase in yolk material. Also, the number of nucleoli increases progressively during oocyte maturation. In addition to the oocyte changes, there are changes in the zona pellucida. The primary follicle lacks a zona pellucida, but one begins to form as a result of the exchange between the oocyte and the follicular cells in secondary follicles, and becomes complete only in tertiary follicles. The follicular cell layer in the primary oocyte is incomplete and consists of only a few flat cells. This layer develops into a complete layer of cuboidal cells arranged around the oocyte in the tertiary follicle.

Atypical Follicles.—Atypical follicles are structures that may be confused with tertiary follicles, but differ from them in several aspects. In these atypical follicles, the yolk is clustered, there is neither a zona pellucida nor a well-organized follicular layer, lymphocytes are present. The nucleus is absent, and there is an increase in the number of irrigation vessels of the surrounding connective tissue. We interpreted these atypical follicles to be atretic follicles that eventually would be resorbed by the organism.

Non-yolked Structures.—These structures are easily distinguished from the developing and atretic follicles by their lack of yolk. Tonutti (1931) and Wake (1968) described similar structures for several species of caecilians, but Tonutti was reluctant to call them corpora lutea owing to the lack of information about their function. Wake (1977) described these structures as corpora lutea, and reported their occurrence in three species of oviparous caecilians and in most viviparous species. Wake reported that the corpora lutea regulates the development of and subsequent secretions from the oviduct in the viviparous species during pregnancy. Recent histochemical studies by Exbrayat and Collenot (1983) on Typhlonectes compressicauda positively identify non-yolked structures as corpora lutea. The non-yolked structures found in T. compressicauda, morphologically resembled the non-yolked structures we found in Chthonerpeton indistinctum.

Fat Bodies.—The fat bodies of C. indistinctum are similar in morphology and histology to those of other species (Wake, 1968). These are nutritional reserve organs, whose main function is to supply the gonads (Noble, 1931). The sizes of fat bodies would be expected to vary seasonally and with the reproductive stages as noted by Wake (1968).

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LITERATURE CITED


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