Ultrastructure of the Oocyte Vitelline Envelope Deposition in the Amazonian Fish *Pseudotylosurus microps* (Teleostei: Belonidae)

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Abstract

The development of the vitelline envelope during the oogenesis of the "needle fish" *Pseudotylosurus microps* was examined by transmission electron microscopy. In the previtellogenic oocytes the vitelline envelope has a bilaminar structure. The inner electrondense layer seem to be deposited by the oocyte, the outer, less electrondense and granulo-fibrillar layer seems to have an exogenous origin. In the vitellogenic oocytes the vitelline envelope acquires a trilaminar appearance. The outer layer before mentioned become gradually compacted and electrondense. In a subsequent step it becomes central by addition of an outer third stratus with loose aspect and low electrondensity. At the same time, the inner electrondense layer thickens inwardly by forming domeshaped protuberances covered by less electrondense cupulas. The thickening of the median electrondense and the outer layer seems to occur by contribution of material arising from large electrondense bodies located in the intercellular spaces of the follicular epithelium. In the mature vitelline envelope the inner thickest layer has been probably produced by the oocytes as a result of apposition of endogenous material over the cupulas, although a participation of exogenous sources could not be discarded mainly at the final part of it deposition. The median layer seem to be co-produced by the oocyte and exogen sources and the outer layer is exogenous in origin. The large electrondense bodies intermingled with the follicular cells give a very peculiar feature to the follicles, but its specific function is unknown.

KEY WORDS: Oocyte, Vitelline envelope, Chorion, Fish

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Introduction

As the oocytes grow they acquire external coverings that are important to intermediate the relationships with the environment before and during fertilization, and in several cases, also during the embryogenesis.

The deposition of these coverings in fishes, have been studied by several authors (ANDERSON, 1967; ANDERSON and SPIELMAN, 1971; DUMONT and BRUMMET, 1980; ABRAHAM et al., 1984; SCHEMEHL and GRAHAM, 1987; CRUZ-LANDIM and CRUZ-HÖFLING, 1989 a, b). According to Ludwig's classification (1874), there are two classes of coverings: the primary covering that are deposited by the oocyte during vitellogenesis and the secondary covering deposited by the follicular cells after the end of vitellogenesis or resulting from the primary covering modification. This late covering may present several types of decorations and/or structures that serve for several purposes including oocyte protection and/or attachment to the substratum (DUMONT and BRUMMET, 1980; CRUZ-LANDIM and CRUZ-HÖFLING, 1993). The primary and secondary coverings of the growing oocytes, together, are called vitelline envelope. These coverings around the mature oocyte will pass to be called chorion (TALBOT, 1981; DUMONT and BRUMMET, 1980).

ANDERSON (1967) recognized three zones in the primary envelope of teleosts: zone 1, first deposited among the oocyte microvilli, zone 2, immediately beneath the zone 1, formed by electrondense granular material; and zone 3, subjacent to zone 2, constituted of amorphous material, organized as a reticular-like network. According to this author, after the end of oocyte maturation, and zones 2 and 3 transformation, the zone 1 degenerates.

This paper explores morphological aspects of the origin and ultrastructure of the vitellins envelope (VE) in the Amazonian teleost, *Pseudotylosurus microps*.

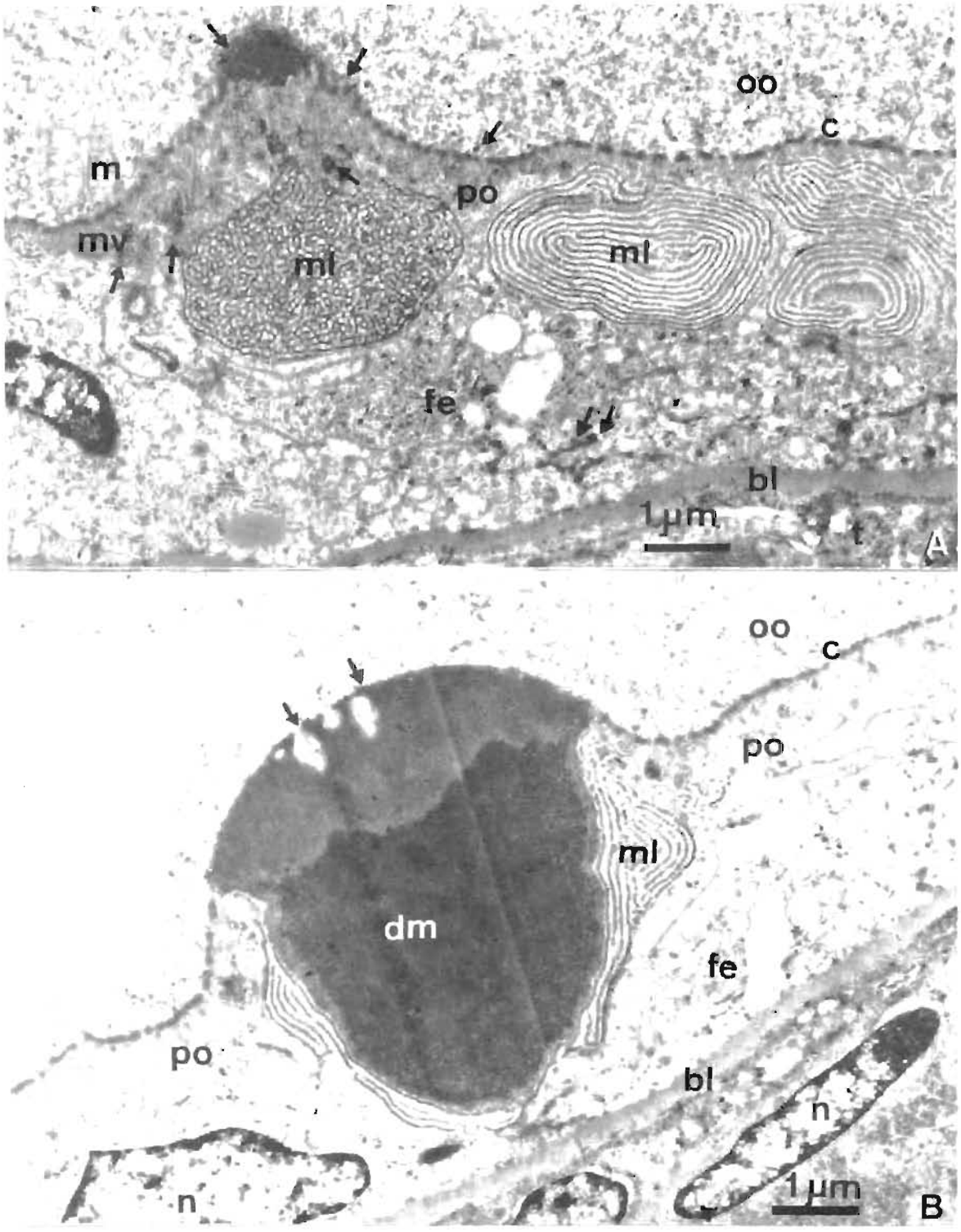

Fig. 1: *Aspects of the material present in the perioocytic (po) space of follicles in the perinucleolar stage.*
A: *First layer of the VE (c) among the oocyte (oo) microvilli (mv) and material with lamelar (ml) structure that seems being incorporated to the oocyte (oo) (single arrows). Notice electrondense material in the intercellular space of the follicular cells (double arrows). bl = basal lamina.*
B: *Dense material (dm) surrounded by lamellar material (ml) compressing inward the oocyte (oo) surface. Notice small portions of cortical cytoplasm of the oocyte into the material (arrows).*
fe = follicular epithelium; n = nucleus; c = oocyte envelope.

Material and Methods

Ovaries from the Amazonian fish *Pseudotylosurus microps* - "needle fish" (Teleostei: Belonidae, Atheriformes, Acantopterygii) were collected, in an Amazonian Expedition through the Solimões River during the breeding season (corresponding to December-March in the Southern hemisphere). For electron microscopy ovaries were fixed in 2.5% glutaraldehyde in 0.2M sodium cacodylate, pH 7.2, buffer, with 4% sucrose, post-fixed in 1% OsO4 in the same buffer, dehydrated in crescent concentration ethanol series and via propylene oxyde embedded in Epon-Araldite mixture. Sections were obtained with glass knives in a Porter-Blum MT-2 ultramicrotome and double-stained with uranyl acetate and lead citrate. The examination were on a Zeiss EM 9S2 electron microscope.

Results

During the perinucleolar stage of the primary growth phase, the oocytes of *P. microps* are surrounded by a closely applied single layer of very flat follicle cells supported by a thick basal lamina covered by the theca. As the oocyte develops, a small narrow space, called perioocytic or perivitelline space, appears between oocyte and follicle cells, into which short microvilli from the oocyte and from the follicle cells are projected.

In the previtellogenic oocytes of *P. microps* the perioocytic space is at first apparently empty. Soon after, substances started to be there deposited, as an electrondense material among the microvilli and closely associated to the oocyte plasma membrane (fig. 1A-B), and represent the primary deposit of the viteline envelope (VE). Subsequently a second layer constituted of a less electrondense material is deposited outward. The components of this outer layer seem to arise from substances which are occupying the sinuous narrow interstices of the interdigitated follicular cells, being though, extrafollicular in origin. Characteristically, the electrondense deposites are dome-shaped thickenings covered by a cupula of less electrondense material (figs. 1B).

The follicle cells concomitantly increase in height, becoming cuboidal and present a nucleus with a prominent nucleolus, mitochondria and some profiles of rough endoplasmic reticulum. At this point there is no signal of secretory activity in these cells, or any morphological indication of material extrusion to the follicular interstices. However, material finely granular, of high electron density is seen in the perioocytic space, and seem to be incorporated by the oocyte (Fig. 1A)

The dome-shaped thickenings, seen in figure 1B, and their cupulas seem to be loci of the VE growing, which later become flattened by a continuous and regular deposition of material at their laterals (compare figs. 1A – 3A). As a result, the intervals among the thickenings slim and eventually disappear giving rise to a VE composed of two layers in the early vitellogenesis. This behaviour is suggestive that the less electrondense cupula material of these inner deposits is produced by the oocyte as well as part of the highly electrondense layer, at least in its initial stage of deposition.

In a second stage an extraoocytic source of material would be responsible for the thickening of this layer. Figure 3B documents the exogenous origin of some components of the VE also at early vitellogenesis. It is likely that this layer previously loose become compacted and highly electrondense as vitellogenesis advances (fig. 3A). A distinct line crossing centrally this layer gives support to this interpretation.

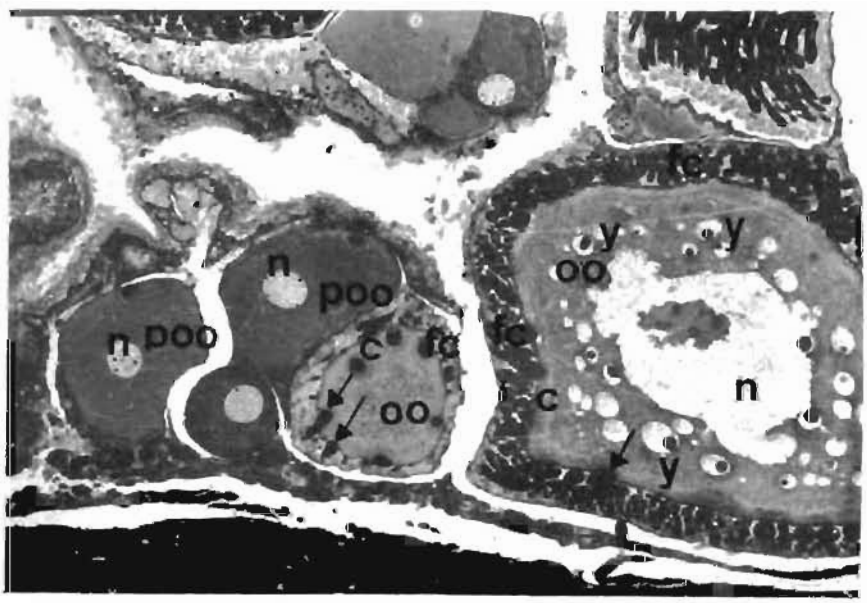

Fig. 2: *Light microscopy micrographs of VE deposition, showing dark stainned material in the follicular epithelium (fc) protunding toward the oocyte (arrows) . n= nuclei; poo = pre vitellogenic oocyte; c = oocyte cytoplasm; y = yolk.*

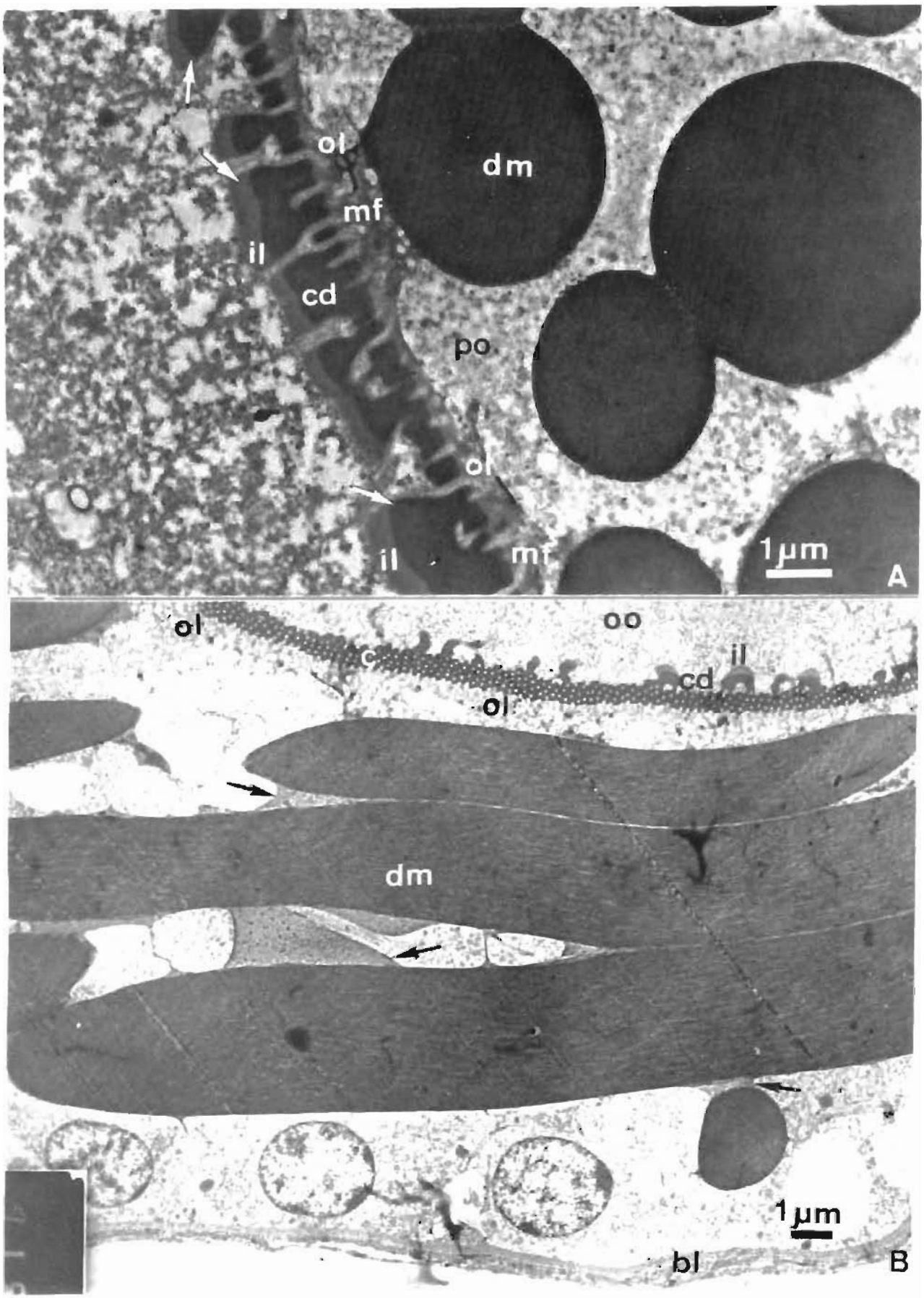

Fig. 3: *Aspects of electrondense material (dm) around the vitellogenic oocytes (oo).*
A: *Contact between the dense material in the perioocytic space (po) with the outer layer of the VE through filamentous material (mf).*
B: Elongated bodies of dense material (dm) showing fibrilar organization. The arrows point to interconnexions of less dense material coming through the follicular cells intercellular space. Notice the basal lamina (bl) darkly doted.
cd = VE dense layer; il = inner layer; ol = outer layer; oo = oocyte.

Later on, yet during the early vitellogenesis, large eliptical bodies start to be found at the widened intercellular spaces of the follicular epithelium (figs. 3B). At light microscopy they are seen as heteromorphic bodies randomly distributed around the oocyte into the interstices of the new columnar follicular epithelium (Fig. 2). At electron microscopy they show high electrondensity and a fibrillar compactation, sometimes arranged with a paracrystalline pattern (fig. 3B). Apparently these elongated bodies continually increase by incorporation of new material coming through the follicle cells intercellular spaces (Fig. 3B).

Figure 3A depicts some morphological indications that the dense material that is adhering to the outer surface of the oocyte VE, arises from "dissolution" of the periphery of the voluminous electrondense masses of the globous bodies. Figure 3A shows one of these bodies applied to the VE and just in the point of direct contact with the VE, threads indicative of dissolution and incorporation to the envelope, can be observed. Direct contact of the elongated paracrystaline bodies with the VE, was never observed.

The latter outer layer of VE is fully deposited in late vitellogenic oocytes (figs. 3A-B) in this phase the perioocytic space has been filled by the heteromorphic dense masses occupying the wide intercellular spaces of the follicular epithelium (Fig. 2).

Discussion

Despite the several studies dealing with the formation of oocytes acellular envelopes or details of its development on fishes (ANDERSON, 1967; WOURMS and SHELDON, 1976; BUSSON-MABILLOT, 1977; DUMONT and BRUMMET, 1980; CRUZ-LANDIM and CRUZ-HÖFLING, 1989a, b; CRUZ-LANDIM and CRUZ-HÖFLING, 1993), the question about the origin of their components remains without an unanimous accordance. However, it appears clear that the trinomy structure/environmental conditions/function, which are known to be highly interdependent factors, would at certain extent interfer with biological phenomena occurring during oogenesis. Thus, it is possible that the diversity of environments, reproductive processes and even the architecture of the oocyte coverings in fishes, would reflect ecletic contribution by the oocyte, the follicle cells or even the oviduct (or other structures), which would be not respectively restricted to the formation of the primary, secondary or tertiary envelopes of the chorion in the mature oocyte as suggested by LUDWIG (1874). In conclusion those diversity would eventually be responsible by the differences found in the literature about the origin of the oocyte acellular coverings (ABRAHAM et al., 1984; WALLACE and SELMAN, 1990; HYLLNER and HAUX, 1992).

The presence of the dense polimorphic masses of material, was discussed in other papers by the authors (CRUZ-LANDIM and CRUZ-HÖFLING, 1993). The exact origin, content and fate of these masses could not be entirely clarifyed, with only morphological approachs and it is possible that some of the material that contact the oocyte surface in early oogenesis, could be vitellogenic proteins, or their precursors since immunochemical studies (HYLLNER and HAUX) indicate extra-oocyte origin of the oocyte envelope. The authors also observed that the material that will constitute the masses seems to reach the follicular interstices by crossing the basal lamina, which appears dotted with electrondense punctuations (CRUZ-LANDIM and CRUZ-HÖFLING, 1993), as if particulate substances were coming throught the basal lamina of the ovarian follicles.

The sequence of the envelope deposition indicates that probably the inner layer is deposited by the oocyte originating from the oocyte cortical granules (GULYAS, 1980) while the central and the outer layers may originate from material constituting the dense masses present in the follicular epithelium, intercellular spaces. According to this view the chorion would be formed by the oocyte (primary envelope) and by foreing substances (terciary envelope). There were not morphological evidences suggesting a contribution of the follicular cells.

Major deposition of the constituents of the vitelline envelope occurs during the vitellogenesis. It is also at this stage that occurs the major growing of the oocyte. This growth is steroid-dependent (HYLLNER and HAUX, 1992). It is possible that the determinant of the vitelline envelope formation, particularly during the phase of vitellogenesis was also under endocrine control.

Activity of the follicular cells in production of the envelope or masses material was not observed

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