

# ULTRASTRUCTURE OF OVARIAN FOLLICULAR EPITHELIUM OF THE AMAZONIAN FISH *Pseudotyllosurus microps* (TELEOSTEI: BELONIDAE): MORPHOLOGICAL AND HISTOCHEMICAL CHARACTERIZATION OF THE INTERCELLULAR DEPOSITS

CRUZ-LANDIM, C.<sup>1</sup> and CRUZ-HÖFLING, M. A.<sup>2</sup>

<sup>1</sup>Departamento de Biologia, Instituto de Biociências, Unesp, CEP 13506-900, Rio Claro, SP, Brazil

<sup>2</sup>Departamento de Histologia e Embriologia, Instituto de Biologia, Unicamp, CEP 13083-970, Campinas, SP, Brazil

Correspondence to: Carminda da Cruz-Landim, Departamento de Biologia, Instituto de Biociências, Unesp, C.P. 199, CEP 13506-900, Rio Claro, SP, Brazil, e-mail: cclandim@rc.unesp.br

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(With 4 figures)

## ABSTRACT

The present paper reports the presence of great quantities of electrondense intercellular material in the follicular epithelium of *P. microps*. The material apparently is uptaken from circulation and enter the follicle through the intercellular spaces accumulating in the epithelial median-apical intercellular spaces and in perioocytic space. The accumulation starts in the early growth of the primary oocyte and proceed until vitellogenesis. The possible chemical nature and function of the deposits are discussed.

*Key words:* chorion, oocytes, oogenesis, electrondense deposits, follicle.

## RESUMO

### Ultra-estrutura do epitélio folicular do ovário do peixe amazônico *Pseudotyllosurus microps* (Teleostei: Belonidae): caracterização morfológica e histoquímica dos depósitos intercelulares

Neste trabalho é mostrada a grande quantidade de material eletrondenso intercelular no epitélio folicular de *P. microps*. Aparentemente, o material é captado da circulação e enviado para o folículo por meio dos espaços intercelulares, acumulando-se nos espaços intercelulares médio-apicais do epitélio e no espaço perioocítico. A acumulação é iniciada no oócito primário e prossegue até a vitelogênese. A natureza química desse material é discutida.

*Palavras-chave:* cório, oócitos, oogênese, depósitos eletrondensos, folículo.

## INTRODUCTION

The ultrastructure of oogenesis in fishes have been studied by several authors concerning the developmental stages of oocytes growth (Anderson, 1974; Iwasaki, 1973; Bruslé, 1980; Cruz-Landim & Cruz-Höfling, 1979; Droller & Roth, 1966; Norrevang, 1968; Wourms, 1976; Shackley & King, 1977), the cellular envelopes of the devel-

oping oocyte (Chaudry, 1956; Cruz-Landim *et al.*, 1987; Nicholls & Maple, 1972; Lambert, 1970; Shelton, 1978; Cruz-Höfling & Cruz-Landim, 1992; Hirose, 1972; Cruz-Landim, 1990; Khoo, 1975) and the oocyte acellular covers, during its growth and after egg maturation (Anderson, 1966, 1967; Busson-Mabillot, 1977; Cruz-Höfling & Cruz-Landim, 1990; Dumont & Brummett, 1980; Hart *et al.*, 1984; Lopes *et al.*, 1982; Wourms &

Sheldon, 1976; Tesoriero, 1978; Fleger, 1977). However although seems to have accordance among the authors about the phases of the oocyte growth and the description of the characteristic structures present in it the same do not happens concerning the egg cellular and acellular covers. In fact the structure of follicular epithelium seems to be very much variable from one group to another. In face of this it function, as well as the function of the thecal sheath is not well established. In the same way the acellular covers that invest the oocyte during it development and after ovulation do not have it origin and function well defined.

These structures have, however, had a long-standing appeal to the biologists since questions as ovary steroid synthesis, and adaptation of oocytes to certain environment conditions have been attributed to the cellular and acellular coverings respectively.

This work intent to contribute to the heap of knowledge about the oocytes covers, when still in the ovary, in order to form a background to the understanding of the pendent questions. In this context, a morphological and histochemical characterization of "adorning" elements found in the interstices of the follicular epithelium was made.

## MATERIAL AND METHODS

Fragments of ovaries of the teleostean *P. microps*, collected in Solimões River (Amazon Basin) were fixed to optical and electron microscopic studies.

For electron microscopy the pieces were fixed in 2.5% glutaraldehyde buffered with 0.2 M sodium cacodilate, pH 7.2, containing 4% sucrose and post-fixed in 1% osmium tetroxide in the same buffer.

After fixation the pieces were dehydrated in increasing concentration series of ethanol and via propylene oxide, embedded in Apon-Araldite resin. Sections obtained with glass knives were double-contrasted with uranyl acetate and lead citrate. The examination and photography were made on a Zeiss EM9S2 electron microscope.

For light microscopy the ovary fragments were fixed in 10% neutral formaldehyde and embedded in paraffin. To the paraffin section were applied histochemical tests (see Table 1) according Pearse (1960) to show acidic and neutral muco-

polyssacharids (also the sulfated ones), reducing group as SH, NH groupments, cholesterol and elastic fibers.

## RESULTS

In the course of the study of *P. microps* oogenesis our attention was driven to peculiar features of its follicular epithelium. The follicular epithelium around the oocytes between the primary phase of oocyte development and early vitellogenesis accumulate in its intercellular spaces numerous polymorphous bodies, in resin sections, stainable with toluidine blue and other acidic stains (Fig. 1). During the vitellogenic phase of the oocyte, the number and size of these structures is such that can even shade the epithelial cells to the light microscopy examen.

The origin and evolution of these material revealed to be difficult to follow, but as the ovary of *P. microps* is of asynchronous type several stages of accumulation can be seen at same time in different follicles.

### Ultrastructure

An attempt of follow the evolution of the material accumulated in the intercellular space of the follicular cells was made taken in account the growth phase of the oocyte enclosed in the follicle. By this approach it can be seen that when the oocyte is in the perinucleolar phase and the follicular epithelium is still flat, the material appears only in the apical face, frequently occupying the space between the oocyte surface and the follicular cell top (perioocytic space) (Fig. 2A, B). In this stage the bodies appear made of reticular or concentric lamellae of electrondense material as in Fig. 2A or as compact electrondense masses (Fig. 2B).

In any case the amount of material accumulated is so, that the perioocytic space is enlarged and the follicular cells and oocytic surface deformed (Figs. 1 and 2).

It seems that the masses are compressed against the oocyte surface, producing an intrusion in its cytoplasm. The microvilli present in the oocyte surface, frequently disappear from these regions and there are morphological evidences that the material could enter the oocyte cytoplasm, as seen in the Figs. 1 and 2A, where the denser part of the material seems to pulverized and pass

to the oocyte cytoplasm or as in Fig. 2B, where the electrondense mass push the oocyte surface inward.

In this phase the follicular epithelium intercellular spaces are note widened but some electrondense material may be seen fulfilling the basal portion of the cellular contacts (Fig. 2A, B).

In vitellogenic oocytes the follicular epithelium have increased very much in height and the intercellular spaces between cells appear totally occupied by polimorphous dense bodies (Figs. 1, 3 and 4).

The now electrondense bodies may appear homogeneously amorphous, and spherical, as in Fig. 3 or elongated and fibrillar, with differently structured material aggregated to their periphery (Fig. 4A, B).

It seems that these bodies tend to coalesce and that the less dense material makes the link between them during the coalescence process. It is not ease to discern the intercellular localization of this material because of its huge size that somewhat disorganized the epithelium. Where is possible to perceive the cellular contacts, as in basal side of the epithelium, they are closed. However even there can be seen electrondense material inside it and in connection with the dense bodies.

In this phase the oocyte has already at least two layers of vitelline envelope around it and sometimes can be seen the contacts between the dense bodies and the outer layer (still in deposition process) of this envelope (Fig. 3).

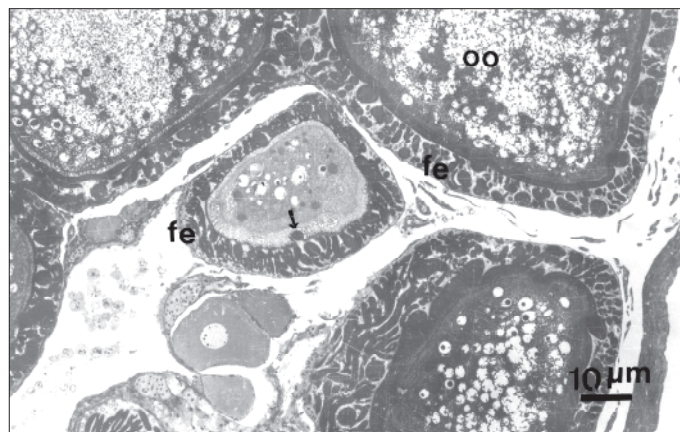
The dense bodies polymorphism may be due to different stages of their accumulation or mobilization. In fact, it can be seen, as already mentioned, the presence of less dense and organized material in the periphery of certain bodies, as well as it can be seen regions where the fibrillar arrangement is patent due both to the loose compactation of the fibrils and to the tangential section of the boundaries of them (Fig. 4A). Some granules present even paracrystalline organization being their fibrillar constituents disposed with highly regular periodicity (Fig. 4B).

The described material starts to accumulate during early perinucleolar phase of the oocyte and probably reaches their peak in early vitellogenic oocytes. However, even after the end of the oogenesis, when the mature egg have already completed the deposition of the acellular coverings, great amounts of the bodies are still present. When, after the end of the oocyte growth the follicular epithelium is disrupted the dense bodies seem remain entire.

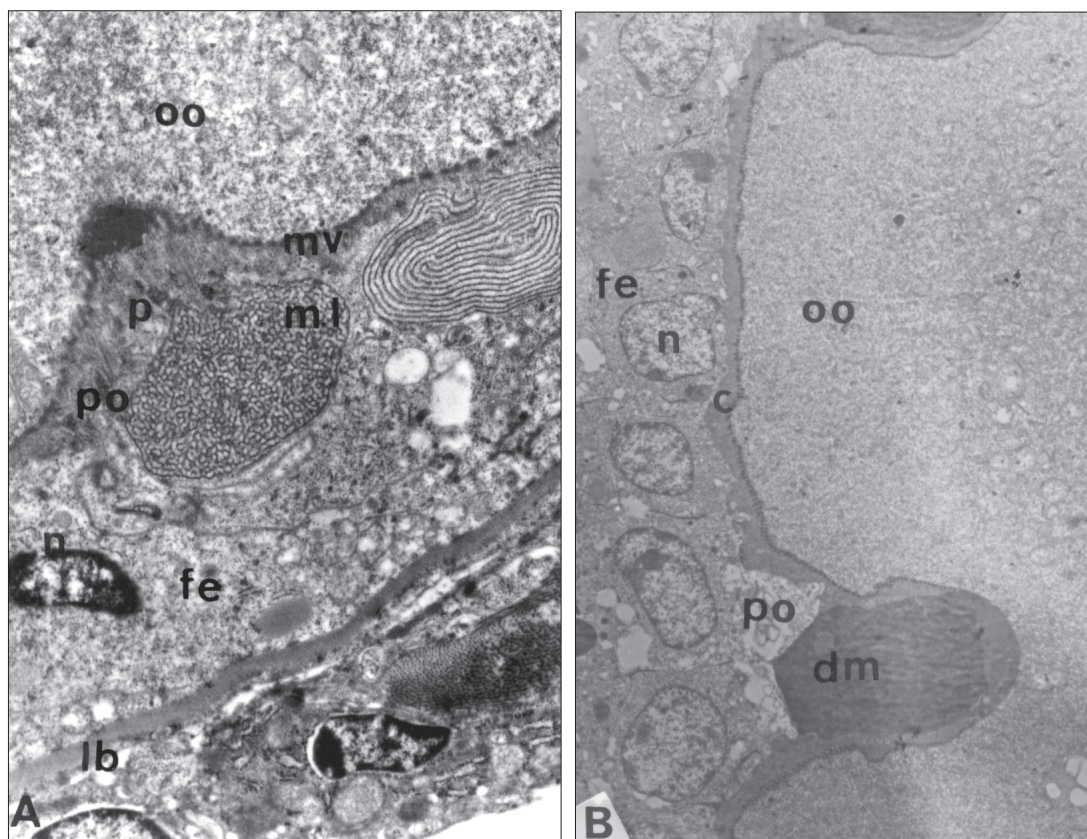
#### **Histochemistry**

As seen in Table 1 the histochemical tests applied to the ovaries show that the material occupying the intercellular space of the follicular cells is negative to Alcian Blue, pH 0.5, 2.5 and Ninhidryn Schiff. It is positive to PAS and Schiff, and react strongly to ferric ferricyanide which indicates the presence of reducing groups.

The previous blokeage by  $HgCl_2$  decrease the reaction intensity, which demonstrates that the



**Fig. 1** — Micrograph of thick a section from material processed to electron microscopy stained with toluidine blue. Oocytes (oo) in different phases of oogenesis showing that in early oocyte development the dense material seem to enter the oocyte (arrows). fe = follicular epithelium.

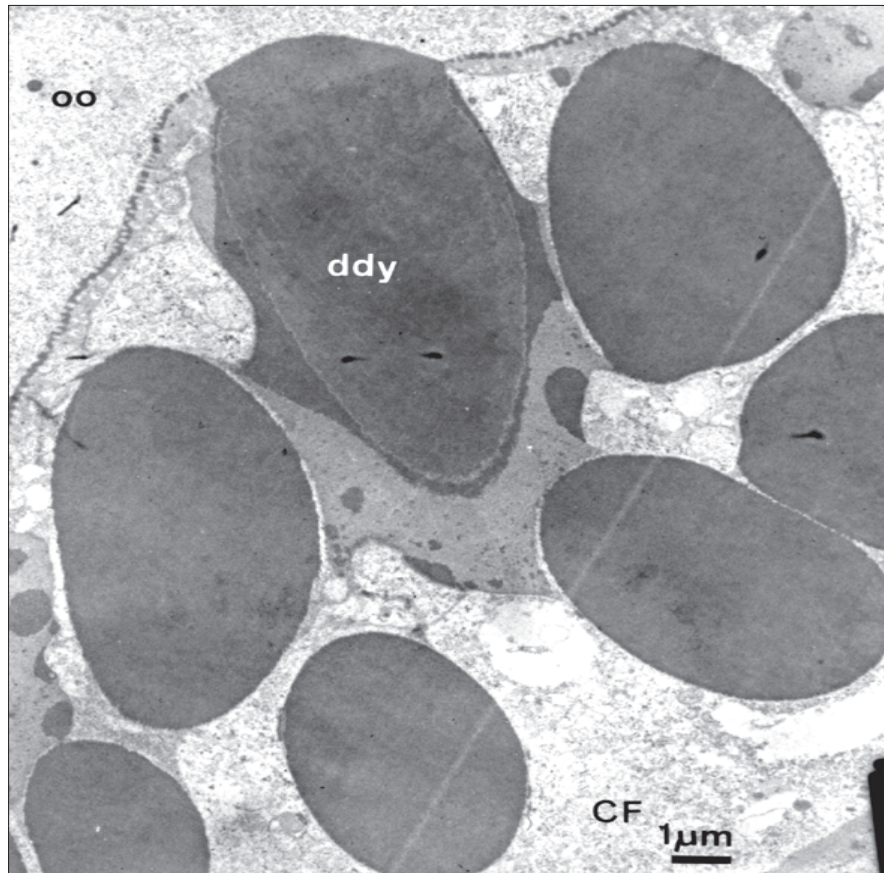


**Fig. 2** — Aspects of the material present in the periocytic (po) space of follicles in the perinucleolar stage. A. Material lamellar (ml) and powder (p) apparently being incorporated by the oocyte (oo). B. Dense material (dm) compressing the oocyte (oo) surface. fe = follicular epithelium; po = periocytic space; n = nuclei; c = oocyte envelope; mv = microvilli; lb = basal lamina.

**TABLE 1**  
Histochemical results.

Reactions	Reactive groups	Reaction intensity
Schiff	Reductor groups	±
Ninhydrin Schiff	NH <sub>2</sub> groups	—
PAS	Polysaccharids	+
PAS + diastase	Acid Neutral	— —
PAS + sodium desoxicolate	Lipids with vic-glicol groupments	—
Toluidin blue	Cholesterol	—
Adams (ferric ferricianide)	Reductor groups	+
Adams + HgCl <sub>2</sub>	SH reductor groups	+++
Alcian blue pH 2.5	Proteoglicans	—
Alcian blue pH 0.5	Sulfated proteoglicans	—
Aldehyde fucsin	Elastic fibers	—

— Negative reaction.  
 ± Weak reaction.  
 + Moderate reaction.  
 +++ Strong reaction.



**Fig. 3** — Contact between the dense material in the perioocytic space with the outer layer of the chorion. Notice the coalescence of the deposits (ddy) by the addition of a new material of different electron densities. CF = follicular cell.

presence of SH groupments, are responsible by the positivity. Also was demonstrated the absence of elastic fibers.

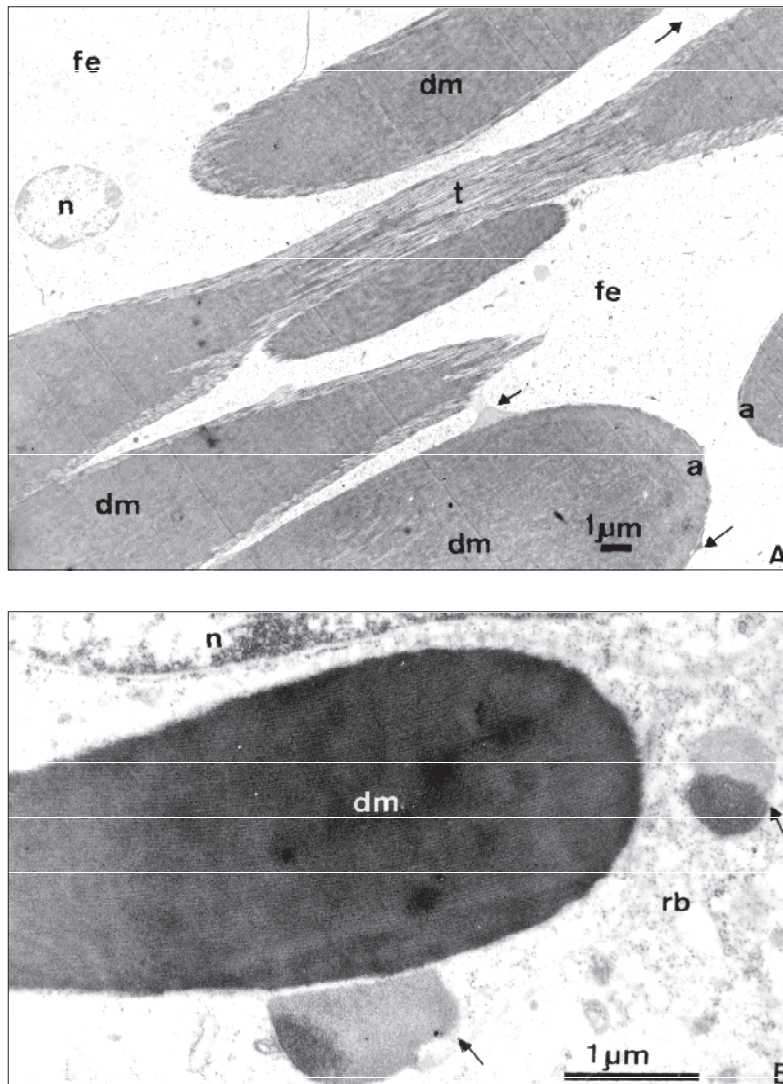
#### DISCUSSION AND CONCLUSIONS

The electron-dense deposits that accumulate in the intercellular spaces of follicular epithelium and in the perioocytic space tend to increase as the oocyte proceeds in its development. This material seems to originate from substances intaken in the basal side of the follicular epithelium and travel to the apical one. The basal intercellular space between cells remains narrow during all the process although filled with electron-dense material, showing that the material must enter this space slowly and accumulate apically. It is clear that the

material present in the perioocytic space reached this location coming through the intercellular space of follicular cells, since in no moment similar deposits were seen inside the follicular cells.

It is known that some or all of the oocyte yolk has an exogenous origin, probably being synthesized in liver and transported to the ovary through circulation (Bruslé, 1980; Droller & Roth, 1966; Norrevang, 1968). The uptake of this vitellogenin material is made through the intercellular spaces of follicular cells and later by pinocytotic activity on the oocyte surface. It is, therefore, possible that some of the electron-dense material, mainly that one arising early in the oocyte development were of vitellogenic nature.

It is also currently known that part of the acellular envelope of oocyte is both synthesized by the oocyte and follicular epithelium (Anderson,



**Fig. 4** — Additional aspects of the dense material (dm). A. Fibrillar nature of the dense bodies (dm) and the amorphous less denser periphery. The arrows point to the less dense material coming through the intercellular space. B. Paracrystalline arrangement of the material in the big masses (dm) and isolated less denser material (arrows). fe = follicular epithelium; n = cell nuclei; rb = ribosomes; a = amorphous material.

1967; Chaudry, 1956; Dumont & Brummett, 1980; Fleger, 1977; Tesoriero, 1978; Begovac & Wallace, 1989). The responsibility more frequently appointed to this synthesis is to the follicular epithelium. However in this case there are some morphological evidence that the electrondense material can contribute to the formation of the outer envelope of the oocyte, or chorion.

The chorion, of mature teleost eggs, is a proteinaceous covering. The paracrystalline pattern

assumed by some of the intercellular bodies seems to point to a proteinic nature, but the Ninhydrin Schiff reaction fail in show the presence of  $\text{NH}_2$  groupments in the material. This fail may be due to the lack of free  $\text{NH}_2$  groups. On the other hand, the presence of SH free radicals in this deposits are not well understood but they were already demonstrated in the chorion of marine teleosteans (Begovac & Wallace, 1989) and in the sea urchin they are responsible by the egg activation.

From the histochemical testes applied only polysaccharids, reductor groups and SH groups was demonstrated present in the intercellular deposits. Therefore also the histochemical results indicate that the intercellular deposits could be formed by vitellogenic or by chorionic proteins.

Whatever are the electrondense material functions, it is strange the great amounts of accumulation and their presence after the end of vitellogenesis is an accessory. However there is a possibility that the remaining material form some sort of appendages, that associated with the egg surface, may serve for eggs fluctuation or fixation to a substrate, as verified in certain species (Anderson, 1966; Hart *et al.*, 1984).

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