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SOME HISTOLOGICAL AND ULTRASTRUCTURAL ASPECTS OF OOGENESIS IN *Piaractus mesopotamicus* HOLMBERG, 1887 (TELEOSTEI)

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ABSTRACT

This paper describes various aspects of oogenesis in *Piaractus mesopotamicus* (pacu), particularly the changes in nuclear morphology and the nuclear-cytoplasmic interactions during the early developmental stages of oocytes. Material produced in the nucleus and passed to the cytoplasm, known as "nuage", was observed in oogonia and in oocytes. In the oocyte cytoplasm, this material gives rise to germ plasm. Vitellogenesis, chorion formation, and atresia of some follicles were also observed and were similar to those described in other teleost fish.

Key words: Nuage, oocyte, oogenesis, oogonia, pacu, *Piaractus mesopotamicus*, teleost

INTRODUCTION

The transformations that occur in oocytes during oogenesis in fish have been divided into at least three stages: (1) nuclear chromatin stage, (2) perinucleolar stage, and (3) vitellogenesis [6]. The main oocyte features used to analyze and characterize these stages are the pattern of chromatin arrangement, the nucleolar organization, the presence and behavior of "nuage", the formation and distribution of cortical granules, the presence and maturation of yolk granules, and the formation of the chorion [1-13,19-21,23-25]. Oogenesis in teleosts includes primary and secondary growth, of the oocyte [17,18,22]. During primary growth, membranous organelles develop and there is cytoplasmic differentiation, with the formation of specialized structures such as Balbiani's body. The secondary growth phase involves preparation for reproduction, with the formation of cortical granules and vitellogenesis that culminates in ovulation [22].

In this report, we describe some of the morphological differentiation that occurs during oocyte development in *Piaractus mesopotamicus* (pacu), a seasonal fish from the Pantanal in the state of Mato Grosso do Sul, Brazil.

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MATERIAL AND METHODS

Female specimens of *Piaractus mesopotamicus* were collected in the Pantanal (Mato Grosso do Sul, Brazil), at breeding and feeding sites in the Rio Aquidauana and Rio Miranda during the months of April and September. The specimens were weighed and measured, and their gonads were removed, measured, and examined for color and ovule content. Fragments of ovary were then fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer and, after two washes in buffer, were post-fixed in 1% osmium tetroxide in the same buffer. Following another rinse in buffer, the samples were dehydrated in a standard acetone series (30% to 100%) and embedded in Epon 812. Semithin sections (1 μm) were examined by light microscopy after staining with methylene blue and azur II. Ultrathin sections (60 μm) were contrasted with uranyl acetate and lead citrate and then examined and photographed in a transmission electron microscope (TEM).

RESULTS

P. mesopotamicus is seasonal and has a cyclic gonadal development. Reproduction occurred in November [16], when the fish had accumulated sufficient energy stores and well-developed gonads weighing up to 364 g each (Table 1). During this stage, the ovaries contained a large number of greenish eggs. In the feeding season, around April [16], the ovaries were a pinkish-orange color and empty (average weight, 15 g) (Table 1). Latecomer fish were shorter and thinner than specimens collected during typical seasons of feeding and reproduction. Latecomer fish also had gonad and body weights that were half of those of other fish collected in the same river. In addition, their gray-colored ovaries were in regression, and appeared to be empty (Table 1).

Table 1. Capture site data and ovarian development of *Piaractus mesopotamicus* (Teleostei).

Capture	Body weight (g)	Body length (cm)	Gonad weight (g)	Ovarian aspect
11/11/1988 Breeding site: Rio Aquidauana	5350	49	364	Greenish with eggs. In reproduction.
3/14/1989 Latecomer fish Breeding site: Rio Aquidauana	2455	42	140	Grayish with some eggs or almost empty. In regression.
3/16/1989 Feeding site (Morro do Azeite): Rio Miranda	2868	42	15	Pinkish-orange and empty. Undeveloped.

Three individuals from each capture were used for the morphological preparations and measurements.

The ovaries of *P. mesopotamicus* were of the synchronic type. Oogonia were always present as reserve primordial germ cells (Fig. 1A) for further reproductive cycles. In a given reproductive cycle, the oocytes in maturation were all at the same developmental stage (Fig. 1A). The classic stages of oocyte development (nuclear chromatin, perinucleolar, and vitellogenesis) were observed.

Oogonia occurred in large numbers in the empty, grayish ovaries of immature and latecomer fish and were arranged in nests at the ovary periphery (Fig. 1A). The oogonia have a large nucleus, disperse chromatin, and a single central nucleolus in which the granular and fibrillar regions were clearly observed (Fig. 1B). Electron-dense material, known as "nuage", and similar to the nucleolar material surrounded the cytoplasmic surface of the nuclear envelope (Fig. 1B). The cytoplasm of the oogonia was rich in spherical mitochondria and polyribosomes. Smooth endoplasmic reticulum (SER) was also observed, mainly at the periphery (Fig. 1B). The spherical mitochondria had few cristae and formed aggregates that appeared to originate through multiplication. These groups of mitochondria were frequently close to the nuages around the nucleus. When distant from the nucleus, the nuage electron-dense material appeared in the center of the mitochondrial aggregates.

Oogonia that undergo the prophase of meiosis are known as oocytes I, since oocyte maturation in the ovary occurs in the diplotene of meiosis I. Oocytes in the perinucleolar stage were found in fish with pinkish-orange ovaries captured at the feeding sites.

When the oocytes started to develop, they usually had a single central nucleolus (nuclear chromatin stage) but immediately afterwards several micronucleoli appeared on the internal surface of the nuclear envelope (Fig. 1A,C). The nucleoli were initially homogeneous and compact, although their surface later began to show signs of material diffusion. The material released from the nucleolus passed towards the cytoplasm through nuclear pore complexes (Fig. 1C and 2A). The transfer of nuclear material to the cytoplasm thus occurred in the oogonium (Fig. 1B) and in the oocyte during the perinucleolar stage (Fig. 1C and 2A,B). The nuages in the oocyte cytoplasm became surrounded by mitochondria (Fig. 2C) to form a spherical complex (Fig. 3A) known as Balbiani's body. The material was then organized into large electron-dense masses that dissolved gradually during dispersal throughout the cytoplasm (Fig. 3B), but was always associated with mitochondria. During dispersion, vacuolization occurred and a web of electron-dense, thread-like material was formed, mainly during the advanced perinucleolar stage (Fig. 3B). At the beginning of vitellogenesis, this material had completely dispersed and was no longer seen in the oocyte cytoplasm. Instead, the latter was now full of free ribosomes. At the end of the perinucleolar stage, large membrane-bound vesicles resulting from the transfer of micronucleolar material to the cytoplasm surrounded the micronucleoli (Fig. 2B). These vesicles collapsed into intranuclear myelinic figures by emptying their vesicle contents (Fig. 2C).

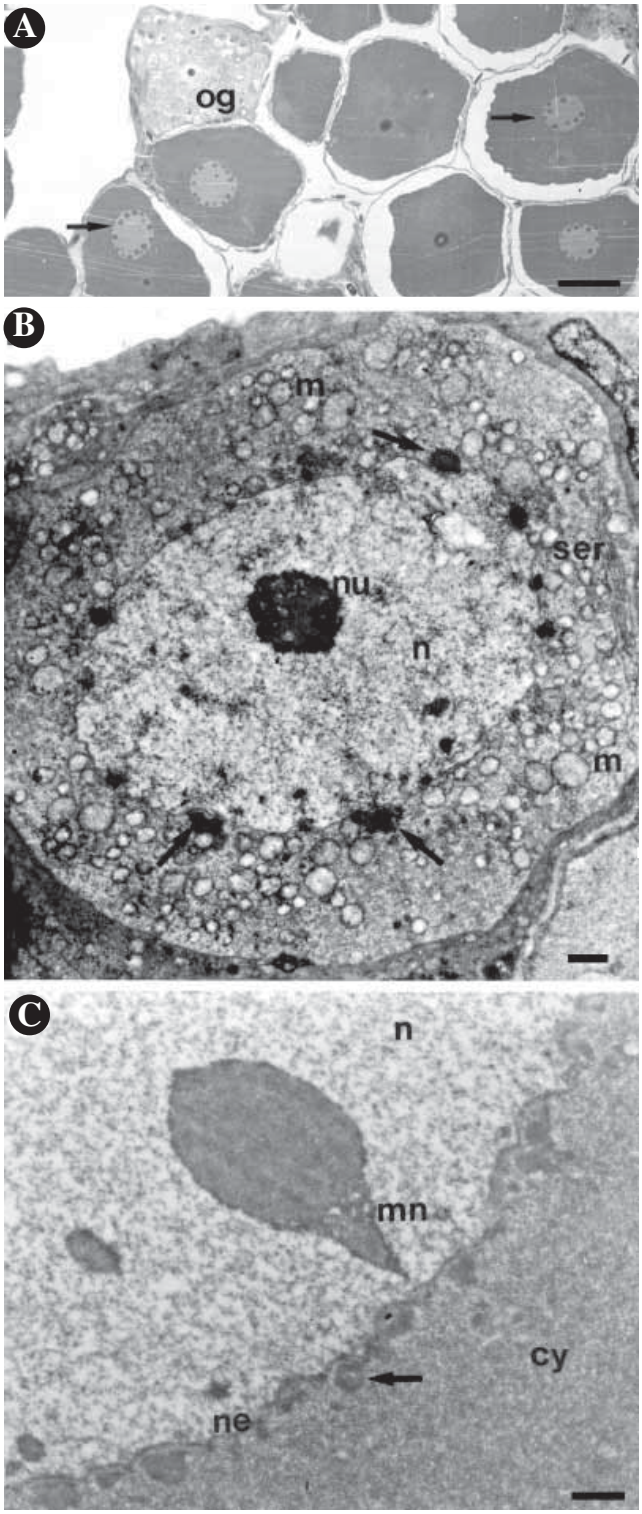

Figure 1. A. Light micrograph showing a group of oogonia (og) in an ovary in the perinucleolar stage (arrows). **B**. Transmission electron micrograph showing an oogonium. Note the large number of spherical mitochondria (m) in the cytoplasm and their association with the material being eliminated from the nucleus (n), and also the formation of nuages (arrows) close to the nuclear envelope. **nu** - nucleolus, **ser** - smooth endoplasmic reticulum. **C**. Detail of a nucleus in the perinucleolar stage with a diffusing micronucleolus (mn). **Arrow** - nuage, **cy** - cytoplasm, **n** - nucleus. Bar: A - 100 μm, B and C = 1 μm.

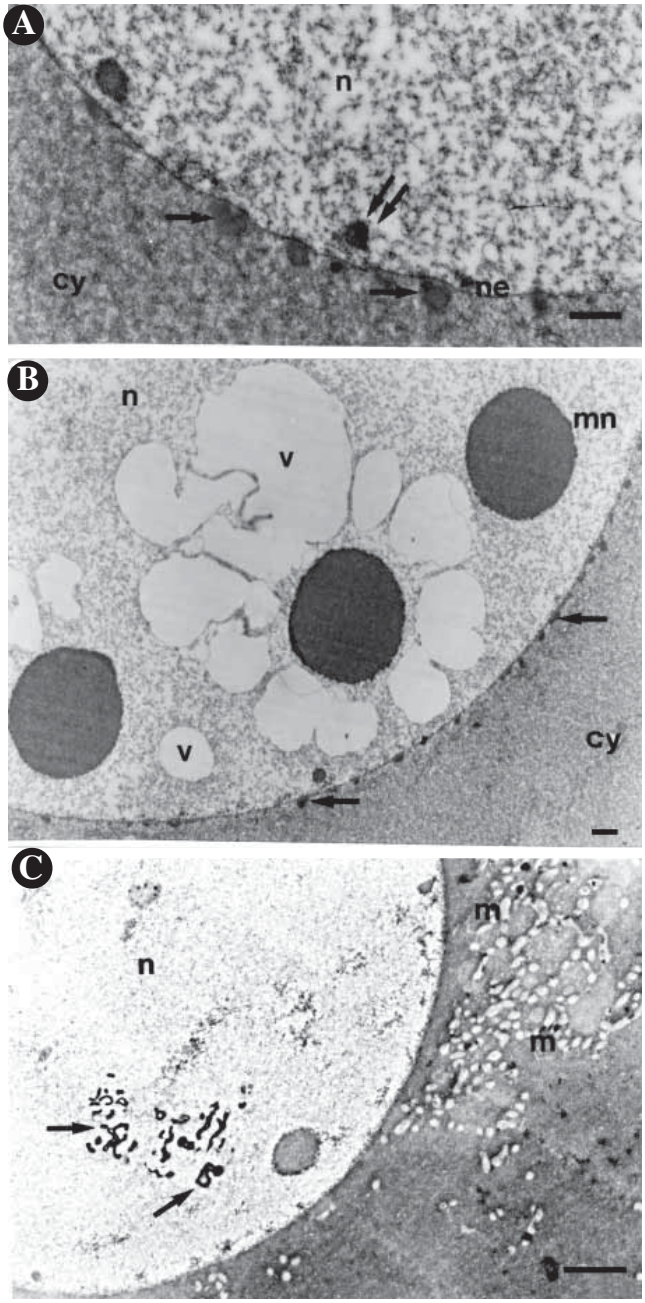

Figure 2. Oocytes during the perinucleolar stage. **A**. Elimination of material from the micronucleolus (double arrows) towards the cytoplasm and the formation of nuage (arrows) close to the nuclear envelope (ne). cy, cytoplasm; n, nucleus. **B**. Vacuoles (v) in the nucleoplasm. Arrows, nuage; cy, cytoplasm; mn, micronucleolus; n, nucleus. **C**. Electron-dense lamellar structures (arrows) in the nucleus (n). Note the association of nuage with mitochondria in the cytoplasm in order to form Balbiani's bodies. A, B and C = 1 μm.

Before the onset of vitellogenesis, the oocyte cytoplasm progressively lost its basophilic nature and the type and number of organelles increased. During the advanced perinucleolar stage, the mitochondria tended to concentrate at the periphery of the oocyte cytoplasm. Annulate lamellae and

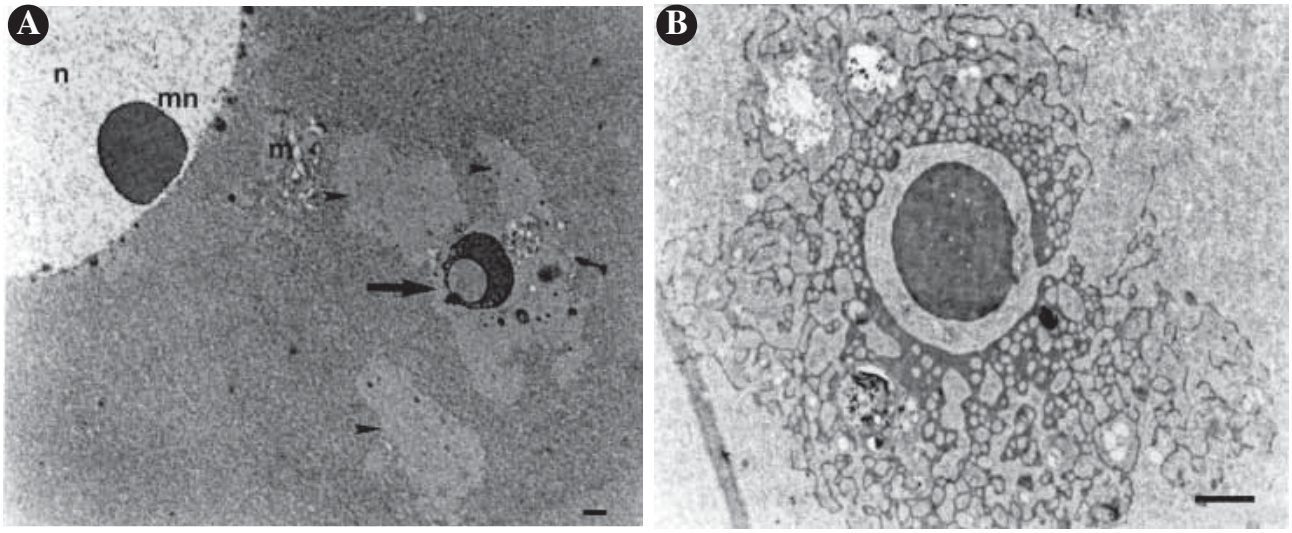

Figure 3. A. Formation of a Balbiani's body (arrow) surrounded by diffuse material (arrowheads) in oocytes in the perinucleolar stage. **m** - mitochondria, **mn -** micronucleolus, **n -** nucleus. **B**. Dispersion of material from a Balbiani's body to form a network of electrondense threads. A and B = 1 μm.

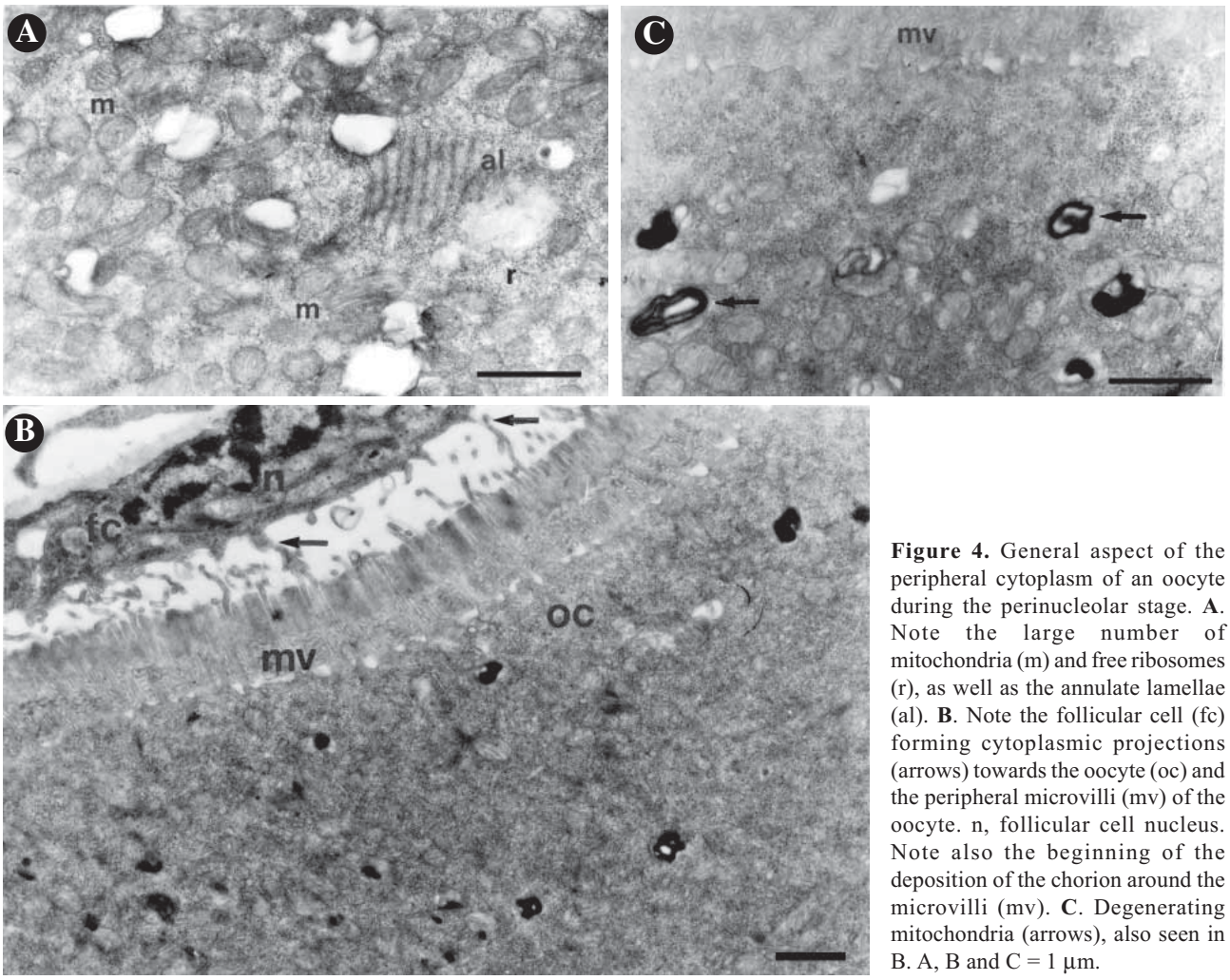

Figure 4. General aspect of the peripheral cytoplasm of an oocyte during the perinucleolar stage. **A**. Note the large number of mitochondria (m) and free ribosomes (r), as well as the annulate lamellae (al). **B**. Note the follicular cell (fc) forming cytoplasmic projections (arrows) towards the oocyte (oc) and the peripheral microvilli (mv) of the oocyte. n, follicular cell nucleus. Note also the beginning of the deposition of the chorion around the microvilli (mv). **C**. Degenerating mitochondria (arrows), also seen in B. A, B and C = 1 μm.

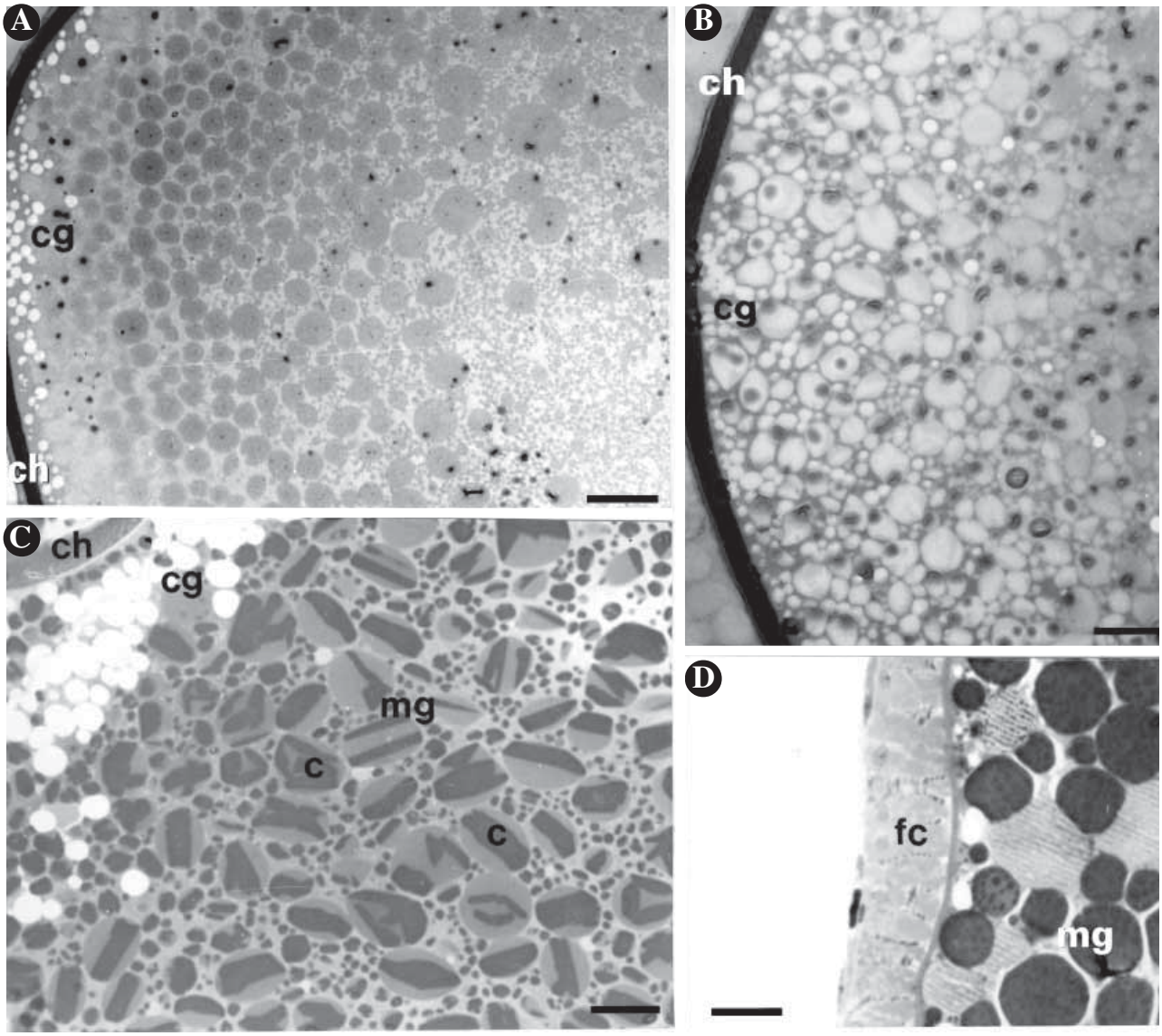

Figure 5. Light microscopy of oocytes in various stages of yolk deposition and chorion production. Notice the presence of cortical granules (cg) in **A** and **B**. Panels **C** and **D** show mature yolk granules (mg), some of which contain crystalloids (c). A layer of follicular cells (fc) is present in D. ch, chorion. Bar = 160 μm in A, and 100 μm in B-D.

myelinic bodies were also seen associated with mitochondria (Fig. 4A-C).

The appearance of electron-transparent granules at the periphery of the oocyte cytoplasm marked the beginning of the vitellogenesis. These granules were often smaller than the granules of the mature yolk (Fig. 5A), and persisted until the end of vitellogenesis (Fig. 5C). Their localization and morphology suggested that they were homologous to the cortical granules of mammalian oocytes. In pacu, the yolk granules initially appear in the center of the oocyte where they form aggregates of small granules that later increase in size as they spread to the cell periphery (Fig. 5A). Immature granules were amorphous (Fig. 5A,B), whereas most mature granules contained crystalloid electron-dense material (Fig. 5C). Vitellogenic oocytes occurred in the ovaries of fish from the breeding site and were much larger in the final stage of vitellogenesis. At this point, the oocyte cytoplasm was full of mature yolk granules (Fig. 5C and 6C). The nucleus/cytoplasm volume ratio was inverted since at this stage the nucleus was very small and was rarely observed in histological sections because the superposition of the yolk. Several very small mitochondria were associated with the yolk granules and the cytoplasm was poor in other types of organelles (Fig. 6C).

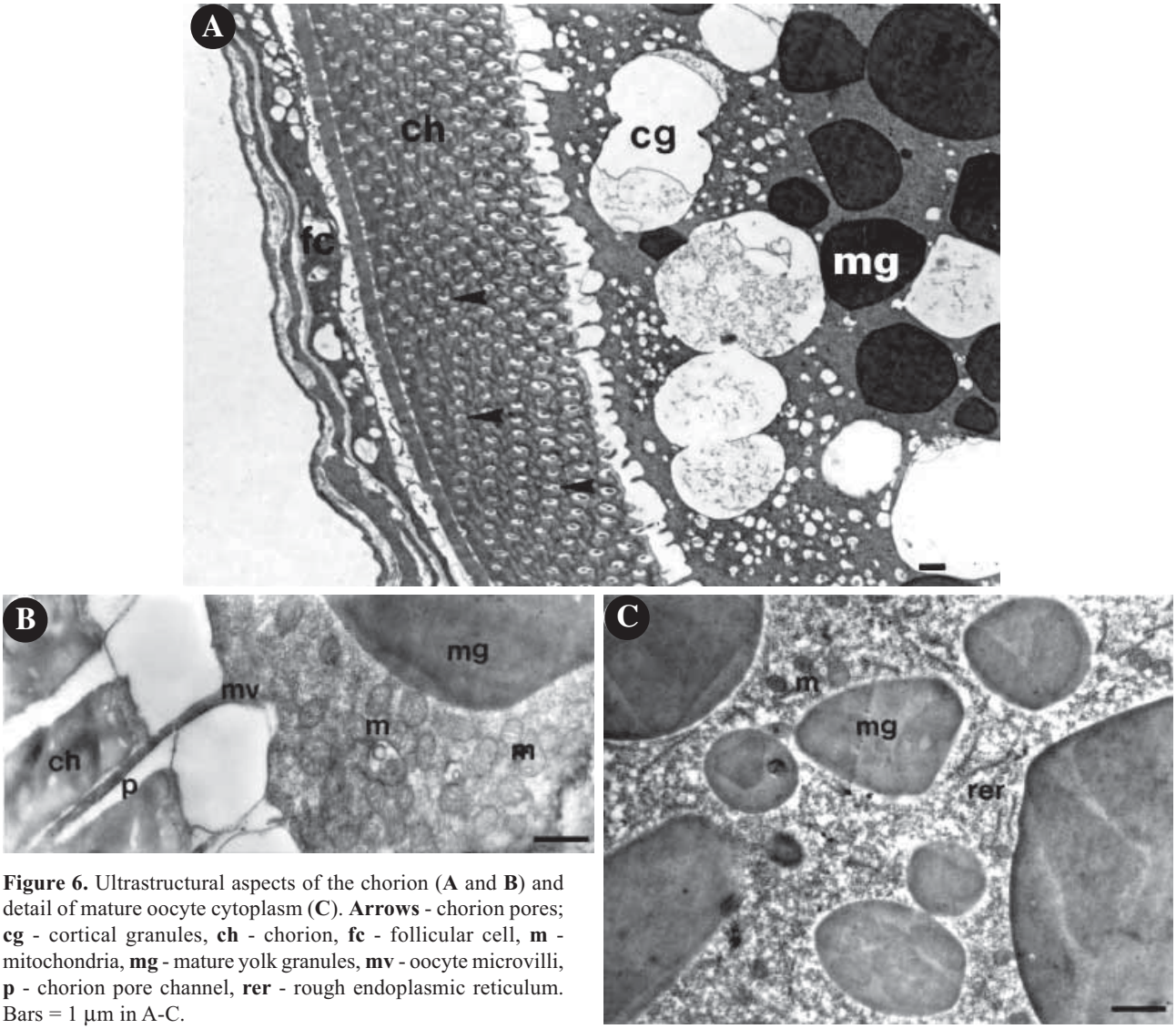

Figure 6. Ultrastructural aspects of the chorion (**A** and **B**) and detail of mature oocyte cytoplasm (**C**). **Arrows** - chorion pores; **cg** - cortical granules, **ch** - chorion, **fc** - follicular cell, **m** - mitochondria, **mg** - mature yolk granules, **mv** - oocyte microvilli, **p** - chorion pore channel, **rer** - rough endoplasmic reticulum. Bars = 1 μm in A-C.

During vitellogenesis, the chorion started to be deposited (Fig. 4B and 6A) by the oocyte (vitelline membrane; Fig. 6B) and follicular cells (Fig. 4B and 5D). Both the oocytes and follicular cells had microvilli in their apical surfaces (Fig. 4B and 6B). The chorionic material was deposited around these microvilli and became perforated by pore-channels when the microvilli retracted (Fig. 6A,B). The cortical granules were still present at the end of vitellogenesis (Fig. 5C and 6A) and may have some role in modifying the chorion following ovulation.

Regression of the ovary after ovulation was part of the reproductive cycle since during this phase the ovary was being prepared for the next cycle of oogenesis. During this stage, the ovaries showed a drastic reduction in volume. In *P. mesopotamicus*, the beginning of follicular involution was characterized by degeneration of the oocyte, with the appearance of several myelinic bodies and a general disorganization of the cytoplasm (Fig. 7). The follicular epithelium was low and interrupted, and the cells were separated from the oocyte. Rupture of oocyte plasma membrane resulted in the release of its content into the interstitium.

DISCUSSION

As in other vertebrates, such as mammals, the stages that precede vitellogenesis in fish occur with no hormonal influence, but subsequent stages of maturation are under the control of the hypophysis [24]. During the initial stages that precede yolk formation, the oocyte looks like a relatively undifferentiated cell that is poor in organelles, except for many mitochondria, and has a basophilic

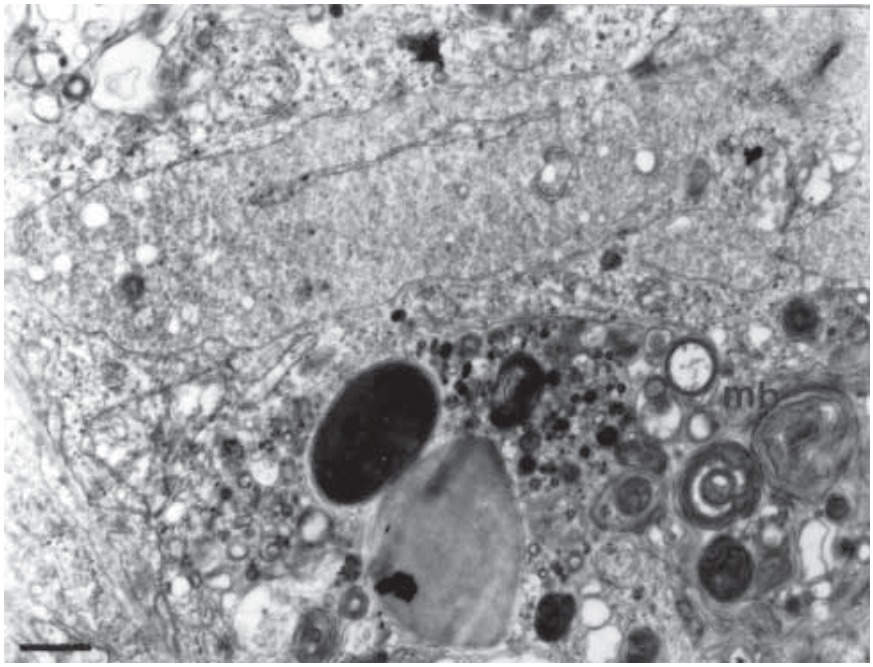

Figure 7. Details of a degenerating follicle. mb, myelinic body. Bar = 1 μm.

cytoplasm, because of the presence of free polyribosomes. During the nuclear chromatin stage, the oocyte nucleus usually has a single, large nucleolus. The perinucleolar stage can be subdivided into two phases and is characterized by the occurrence of micronucleoli around the nucleus. At the beginning of this phase, the oocytes are poor in organelles and are bosophilic. The advanced perinucleolar phase is characterized by the progressive decondensation of the micronucleoli, the transfer of their material to the cytoplasm, the formation of Balbiani's bodies, the loss of cytoplasmic basophily, and the diversification of intracytoplasmic organelles [7]. In *Astyanax bimaculatus* [6], the cortical granules first appear at the end of the advanced perinucleolar stage, which marks the beginning of yolk formation. At the end of this stage, the chorion starts to form [8]. During yolk accumulation, which is controlled by the hypophysal hormones, yolk granules appear in the oocyte cytoplasm and greatly increase the cell size. These granules apparently have two origins. One is exogenous, via the absorption of substances from the interstitial fluid, mainly through the intercellular spaces of the follicular cells, and the second one is endogenous, through synthesis in the oocyte itself [9,10,19,21].

Although the ovaries of *P. mesopotamicus* were of the synchronic type, the co-existence of a few oocytes in the initial stages of development alongside mature oocytes was observed. The oogonia developed into nests generally located at the periphery of the ovary. All of the typical stages of fish oogenesis [6] were observed in wild and cultured *P. mesopotamicus* and did not differ from those described in the literature.

No intake of exogenous material was observed in *P. mesopotamicus*. The fact that the yolk granules appeared primarily in the central region of the oocyte suggested that their origin was endogenous, in contrast to other species of fish [4,9,13,19]. The observation that some chorion layers had already begun to be deposited before the yolk first appeared, supports this interpretation. However, the oocyte apparently did not possess the cytoplasmic structures, such as rough endoplasmic reticulum and developed Golgi complexes, required for protein biosynthesis. These compoments are present in yolk, as judged from crystallization of the latter a common phenomenon in granules consisting of almost pure protein [15]. Immediately before vitellogenesis, several arrays of annulate lamellae originate in the oocyte cytoplasm. These structures are believed to be formed from the nuclear envelope and would promote an increase in the amount of endoplasmic reticulum [15].

The transportation of electrondense material from the nucleus to the cytoplasm occurred in two stages of oogenesis (in oogonia and in the oocyte), and was associated with mitochondria in both cases. Material

with these same characteristics has been identified in several species of vertebrates and is designated as "nuage" because of its irregular shape and the presence of dense spots or "clouds" in certain regions of the cytoplasm. The function or destination of this material in oogonia remains unknown. However, there are indications that in the oocyte this material forms the germ plasm responsible for the germ line of the next generation [7,14].

The cortical granules were formed before the yolk granules and concentrated exclusively at the periphery of the oocyte. Contrary to observations in *A. bimaculatus* [8], these granules persisted in the mature oocytes and may have the same function as in other vertebrates, participating in the hardening of the chorion or zona pellucida after fecundation, thereby preventing polyspermia.

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