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OOGENESIS IN *Sparus aurata* L.

Maria Alice Ramos

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OOGENESIS IN *Sparus aurata* L.

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ABSTRACT

The evolution of gonad activity in *Sparus aurata* L., a hermaphrodite protandric species, is dependent on the age and growth of the fish. In the most part of the one-year-old population, sperm reabsortion is followed by oocyte maturation. The dynamics of oocyte development and related sequential cytological events were followed in this study. The characteristics of the oocyte during the first meiotic prophase are described using electron microscopy technic. Ultrastructural modifications of the nucleus and cytoplasm of the oocyte were found to be linked to the different stages of secretory activity, and with transport and incorporation of vitellogenin by the oocyte. The existence of endocytic compartments and a highly specialised cortex allows the internalisation of vitellogenin. The present study indicates that oogenesis in *Sparus aurata* like in most vertebrates depends on the structural evolution of the organelle connected with the endocitic activity of the cell. During maturation and ovulation, the oocyte contains an enormous amount of reserves stocked as macromolecules, which serve as reserves for later utilisation by the embryos.

Keywords: Teleosts, oogenesis, ultrastructure, nucleus, endocitosis

RESUMO

Título: OOGENESIS EM *Sparus aurata* L. A evolução da actividade da gónada na dourada *Sparus aurata* L., uma espécie hermafrodita, protândrica depende da idade e do crescimento dos animais. Na maior parte da população com um ano de idade dá-se a reabsorção dos espermatozoides seguida pela maturação dos oócitos. A dinâmica do desenvolvimento oocitário e os acontecimentos citológicos durante a profase meiótica são descritos neste trabalho utilizando microscopia electrónica. As modificações ultraestruturais do núcleo e do citoplasma do oócito parecem estar ligadas com os diferentes estados da actividade secretora e com o transporte e a incorporação da vitelogenina pelo oócito. A existência de compartimentos endocíticos e um córtex altamente especializado permitem a internalização da vitelogenina. Este estudo indica que a oogénese em *Sparus aurata*, como na maior parte dos vertebrados depende da evolução estrutural dos organelos relacionados com a actividade endocítica da célula. Durante a maturação final e a ovulação o oócito contém uma enorme quantidade de reservas acumuladas como macromoléculas para utilização posterior pelo embrião.

Palavras chave: Teleósteos, oogénese, ultraestrutura, núcleo, endocitose

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INTRODUCTION

The hermaphrodite protandric species, *Sparus aurata* L., a marine teleost spawning pelagic egg, presents particular aspects of sex determination and gonad differentiation. The sex reversal process inhibits the fertility of females, and only older animals are functional females. The fecundity of females is limited by the evolution of oogonia to oocyte maturation and ovulation.The morphological changes occurring in the oocyte during the meiotic prophase and the dynamic aspects of its growth, were used to indicate the receptivity of the oocyte to external factors that can induce final maturation and ovulation. After the reabsorption of the spermatozoa by the Sertoli and epithelial cells, during the second or third year, part of the population become functionally female (Zoar *et al*., 1978). In fact, only these phenotypic females can synthetise the yolk protein precursor internalised in the oocyte by receptor-mediated ligants (Goldstein *et al*., 1982). This gonadotrophic-dependent phase takes place after the structural evolution of the oocyte organelle during the first meiotic prophase (Anderson, 1967; Lam, 1982; Bruslé and Bruslé, 1983).

MATERIAL AND METHODS

508 specimens of *Sparus aurata* were collected in the Algarve, and in the Óbidos lagoon using trammel nets in different months of the year. Age determinations were made by direct readings of the scales. The fork length (cm) and weight (g) were correlated with age. Specimens with different ages were collected from the wild (using trammel nets) maintained, in laboratory conditions, killed and selected organs used for histological study. After macroscopic observation, the gonads were sectioned into pieces measuring 0.5 cm in diameter, these tissues were prepared for light microscopy, to determine the distribution of male and female germinal tissues. Small pieces of the same gonads were (fixed in 3 % glutaraldehyde, sodium cacodylate 0.1 M and 0.05 $CaCl_2$, for three hours, rinsed in buffer (cacodylate) and post-fixed in 1% osmium tetroxide 1h for dehydratation) for electron microscopy studies. The tissue was embedded in Epon. The thin sections were stained with uranyl acetate followed by lead citrate and were examined with a transmission electron microscope.

RESULTS

Physiological state of the oocytes

The nucleus

In the germinal epithelium of *Sparus aurata* L., oogonia with a compact nucleolus originate mitotically oocytes at first meiotic prophase. A basement membrane and rare follicle cells involve the leptoten-pachyten oocyte. First the nucleus presents a chromosome pairing appearance, the synaptonemal complex (Sc) (Fig. 1). Later the nucleolus establishes specific relations with the nucleolar organiser region (NOR) (Fig. 1). At diplotene the nucleolus enlarges, presenting a central fibrilar core and a granular periphery. Multiple nucleoli, in number of twenty, disconnected from the nuclear envelope are nucleolus with an outer granular layer and granules dispersed in the nucleoplasma, or associated with nuclear pores are observed. At late dictyate (Fig. 2) the oocyte contains a nucleolus that has ceased growing and has decreased in size. Some spherical nucleoli containing vacuoles remain at the periphery.

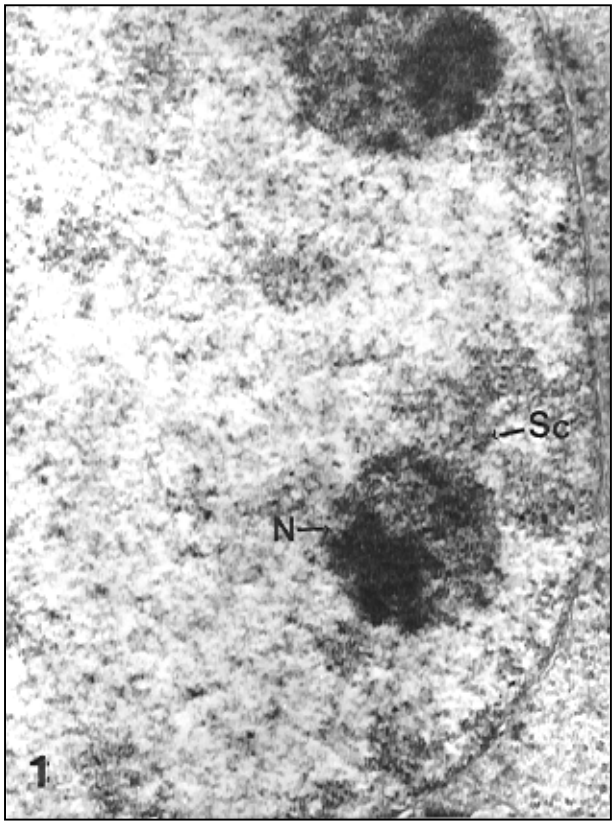

Figure 1 - Leptoten-Pachitene-oocyte. Nucleolus (N) associated to a cromossomal region in proximity to a Synaptonemal complex (Sc). Nuclear organiser region (NOR).120000x.

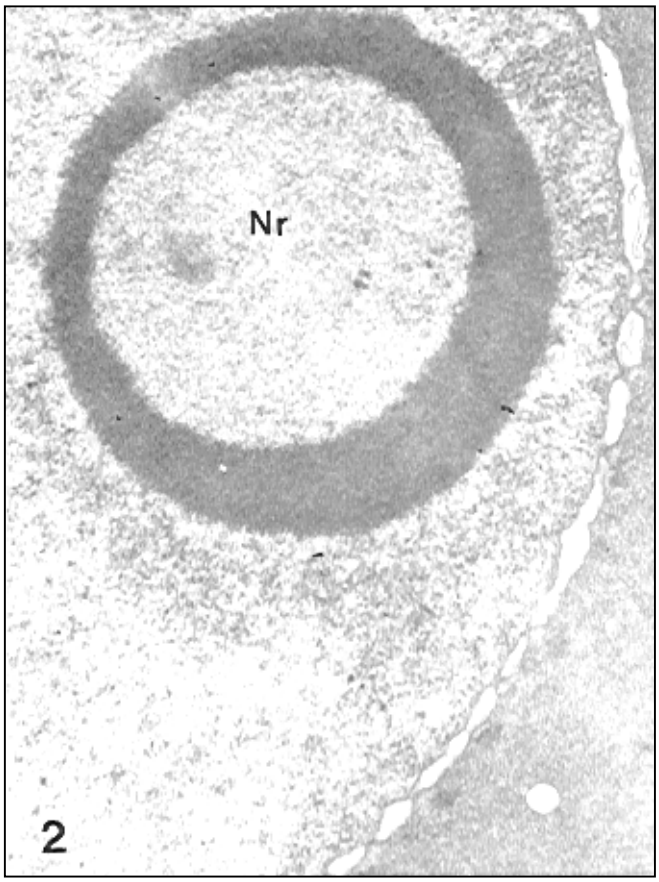

Figure 2 - Diploten nucleus shows a ring shape (Nr) 36000x.

Nucleolus-cytoplasmic interaction

The RNA processing takes place during the migration of the molecules from the site of synthesis, crosssing the nuclear envelope and accumulated in the large number of ribosome, which appear in the cytoplasm before endocytic accumulation of yolk. Granulo fibrilar mass, nucleolus-like bodies (NLB) were observed in all stages of oogenesis in *Sparus aurata* (Fig. 3).

The cytoplasm. Endocytic activity

The endocytic activity in three to four year old females takes place in the oocyte, after RNA accumulation. Endoplasmic reticulum vesicles and elongated mitochondria are dispersed in the cytoplasm, occasionally associated with microfilaments. Golgi stacks are predominantly in a peripheric position near multivesicular bodies (MVB). Lysosome appears in the cytoplasm. Lipid and cortical alveoli are elaborated. Microvilli originated by protusion of the oocyte oolema in the interfollicular space and the microvilli of cells completely surround the oocyte (Fig. 4).

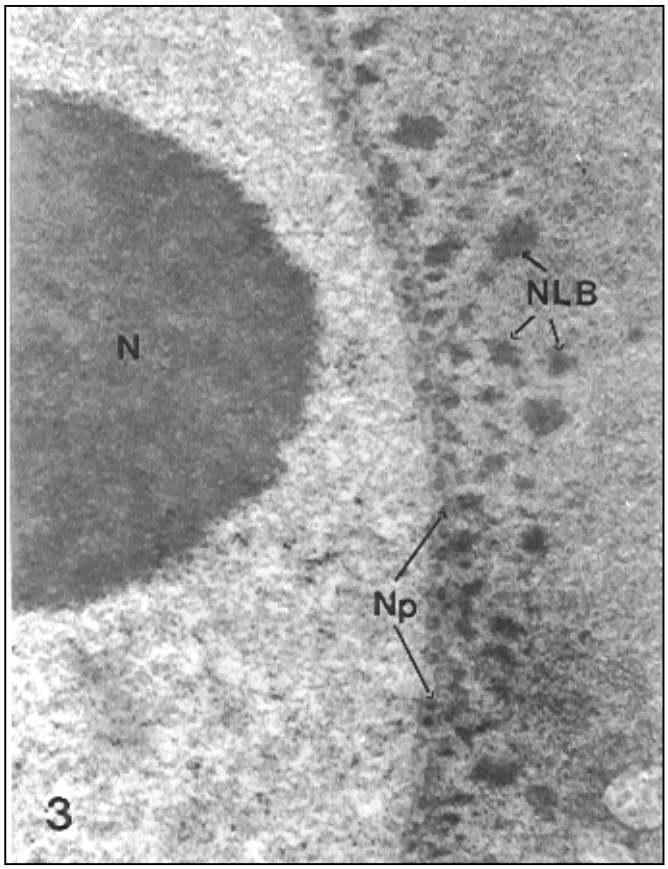

Figure 3 - Nucleus (N). The nucleolus-like bodies (NLB). Nuclear pore (Np). 36000x.

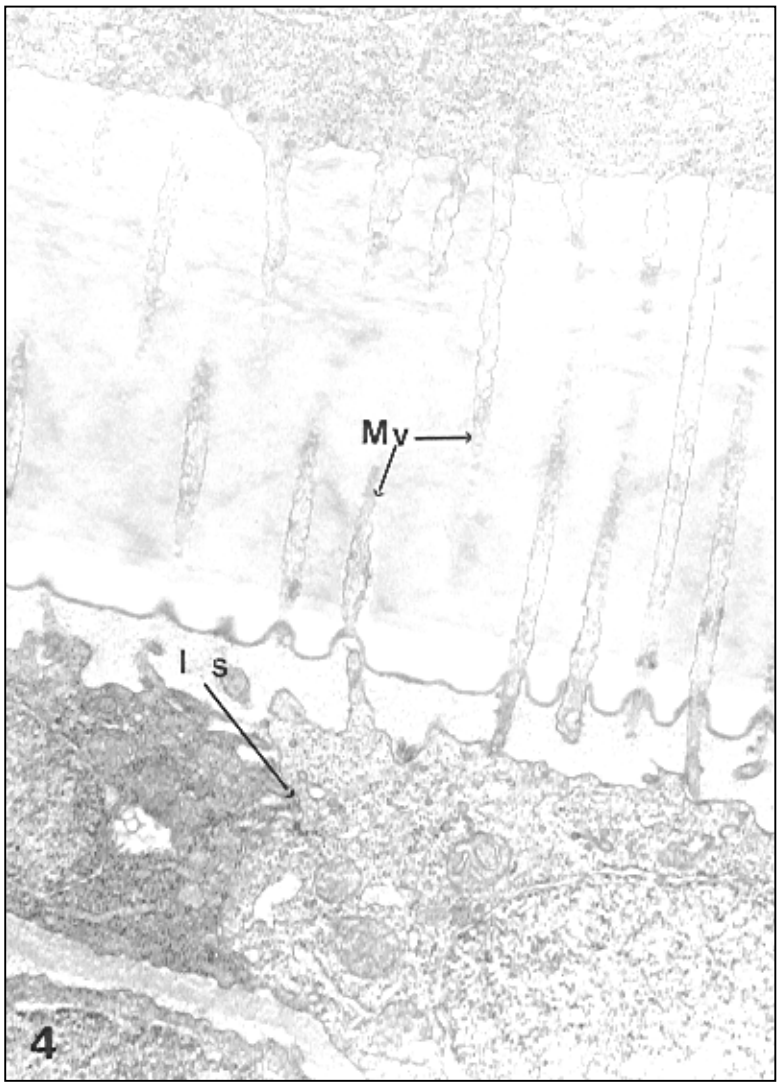

Figure 4 - Microvilosities (Mv). Interfollicular space (Is) .30000x.

The different layers of polysaccharides deposited between the pre existent micro vilosities form the zone pellucid. Microfilaments of 6 nm are observed in the cytoplasm. Pinocytosis is initiated at clathrin coated regions of microvilli which pinch off to form coated vesicles (Fig. 5). These vesicles lose their coats and deliver their contents at endosomes. These transfer the content to the lisosomal compartment and after hydrolysis form the yolk spheres (Fig. 6).

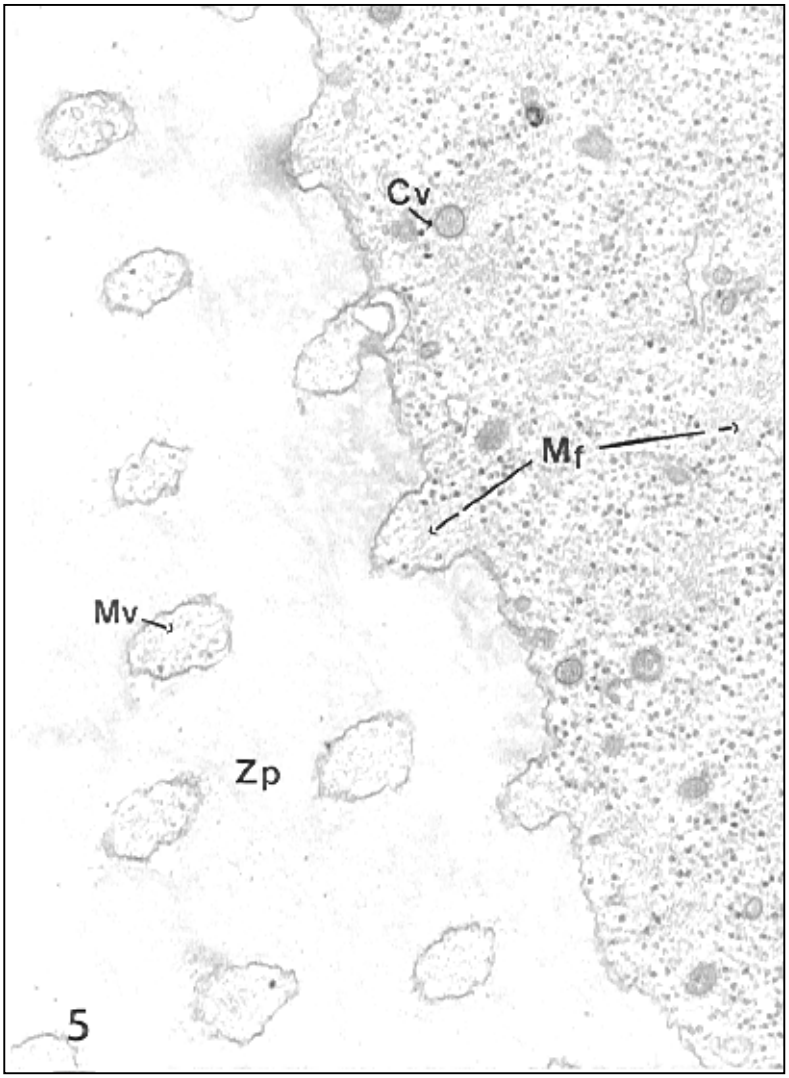

Figure 5 - Coated vesicles (Cv). Microfilaments in the cytoplasm (Mf). Microvilosities (Mv). Zone pellucide (Zp).40000x.

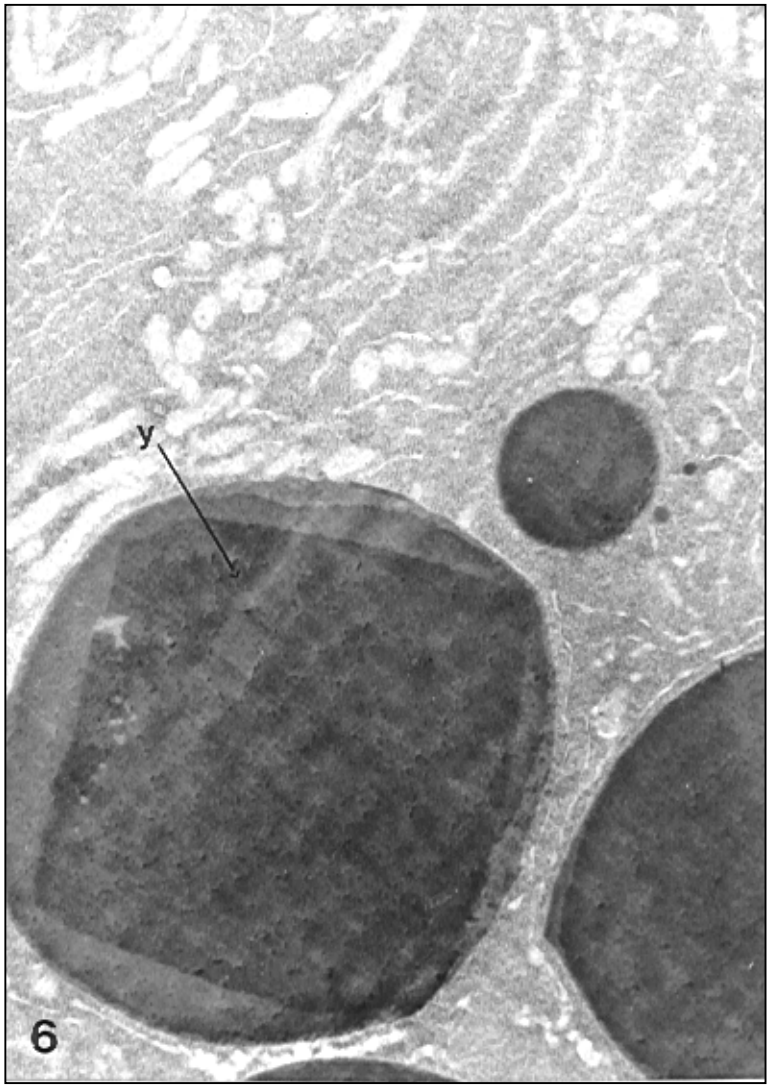

Figure 6 - Yolk spheres are observed. (Y) 16000x.

After using HCG stimuli at 18-20 °C the vitellogenesis is completed in a few hours. The full grown oocyte shows the germinal vesicle excentraly located. The nuclear envelope disrupts after forming several infoldings. Following metaphase II the microvilli are in reabsorption (Fig. 7), the follicle cells degenerate and disperse from the zone pellucida. The yolk components are agglutinated and in a continuous (Fig. 8). After hydration, ovulation occurs and oocites float in seawater prepared for fertilization.

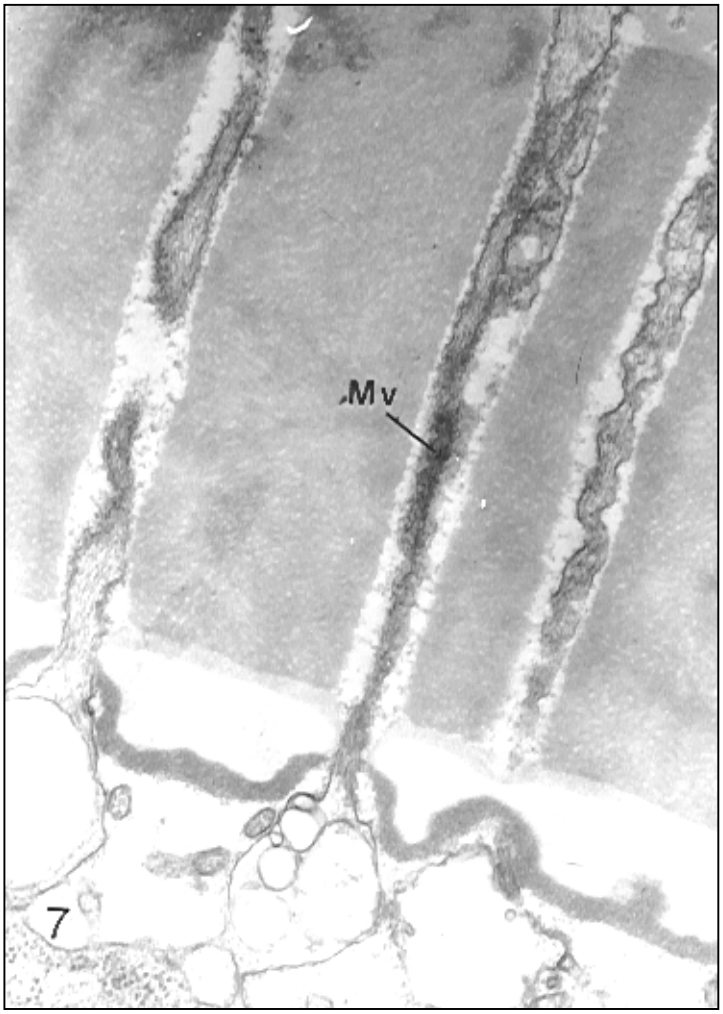

Figure 7 - Microvilli (Mv) are observed 40000x.

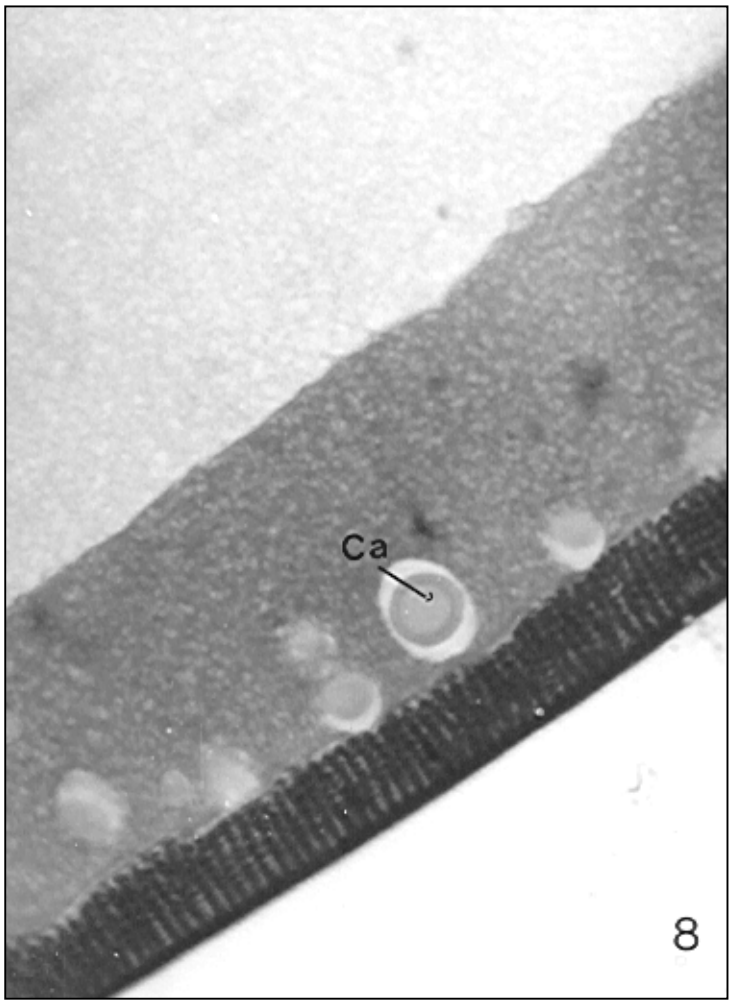

Figure 8 - Cortical alveoli (Ca) are at the ocycite periphery. 1600x.

DISCUSSION

After leptotene-pachitene in the oocyte evolution, it occurs specific relations of the nucleolus with the the NOR that contains the rDNA with transcriptional units for the synthesis of 18s and 28s ribosomal RNA (Goessens, 1984). The RNA synthesis and accumulation is possible due to the amplification of the genes responsible for the rRNA organisation and it takes place during oocyte growth. The protein synthesis for posterior embryonic differentiation depends on the existence of a large number of ribosomes and RNA transfer (Denis, 1977). The RNA 5s is produced during the first growth, which corresponds to 75-80 % of the ribosomic content of the primary oocyte. During vitellogenesis the oocyte mainly produces RNA 28s (Denis, 1977). The nuclear envelope has the function of compartmentation in space during the different stages of the oogenesis, and separates transcription of protein synthesis. Associated with spherical mitochondria, in leptoten-pachytene the NLB has been identified cytoplasmicaly in other fish species as ribonucleoprotein with nuclear

origin (Toury *et al.*, 1977; Azevedo, 1984). At diplotene, the NLB is perinuclear, but mitochondria are dispersed in the cytoplasm. The endocytic (Carmo *et al.*, 1999; Mata, 1999) activity of *Sparus aurata* oocyte depends directly on the structure evolution of the organelle connected with metabolic activity, and on the differentiation of the zone pellucida, follicle and theca cells. It depends indirectly on the synthesis of phospholipoproteins by the liver and its transport and incorporation into the oocyte. A functional three to four year old female with an asynchronous ovary presents oocyte maturation according to a circadian rhythm. The diplotene dictiate in *Sparus* oocytes has a long duration (Ramos, 1986). The one to two year old fish do not enter into vitellogenesis, at least the accellular layers of zone pellucida are not deposited, and probably the follicule cells do not yet synthesise the oestrogen necessary for stimulation of the liver to produce the yolk protein precursor (Aida *et al*., 1973). The oocyte morpholoy of older fish shows an highly specialised cortex and the existence of endocytic compartments which at this stage, allows that the vitellogenin to be taken in large amounts (Routh and Porter, 1962; Goldstein *et al.*, 1982; Mabillot, 1984; Selman and Wallace, 1982) passing through the intercellular space of the follicular epithelium (Abraham *et al.*, 1981). The present study indicates that the *Sparus aurata* oogenesis, like most oviparous vertebrates, depends on the structural evolution of the organelle connected with the auxocitosis and with the endocytosis (Brodsky, 1988) of the cell. At maturation and ovulation, the oocyte contains an enormous amount of reserves stocked as macromolecules, for later utilisation by the embryos.

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