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A HISTOLOGICAL STUDY OF THE ZONA RADIATA DURING LATE OOCYTE DEVELOPMENTAL STAGES IN THE CASPIAN SEA MUGILID, *Liza aurata* (RISSO 1810)

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ABSTRACT

Many functions have been attributed to the zona radiata (ZR), a porous layer normally found between the follicular cell layer and the oolemma in fishes. The ZR has frequently been considered as an interface that regulates the movement of essential material from granulosa nursery cells to oocytes, particularly during vitellogenesis. The ZR also anchors oocytes to substrates via processes extending from this layer. The aim of this study was to examine ZR growing characteristics during the late stages of oocyte development in the marine teleost *Liza aurata* (golden mullet). Specimens of *L. aurata* in the stage IV of ovarian development were caught using beach seines and were injected with an extract from carp hypophysis to induce further development. The porous nature of the ZR was found to exert an increasing role in sustaining oocytes. No ZR was seen by light microscopy in the previtellogenic (II) stage, but following transition to the early stage III, i.e., early vitellogenesis, the ZR appeared in the form of marginal striations. The ZR developed mainly during the late stage IV of ovarian growth, when vitellogenesis was about to end. During development of the oocyte, the ZR is extended as primitive projections towards the inner surface which became more prominent as vitellogenesis progressed. In late vitellogenesis (late stage IV or early V), these projections grew and formed slender finger-like structures arising from follicular cells. The ZR was highly perforated and served as communicating way to the outside of the oocyte. This structural organization accounts for the key role of the ZR in egg development.

Key words: *Liza aurata*, vitellogenesis, zona radiata

INTRODUCTION

Fish oocytes generally have three distinct layers, namely, an outermost follicular layer, a median zona radiata (ZR) and an inner oolemma or oocyte plasma membrane. The follicular layer consists of an outer theca and an inner granulosa layer. The theca has a protective function whereas the granulosa layer is involved in various functions, such as aeration, nourishment of developing oocytes and embryos, secretion of enzymes that participate in the lysis and/or re-organization of certain zones of the egg chorion, transfer of small molecules to the oocytes via communicating junctions, vitellogenesis, steroidogenesis and, in some fishes, synthesis of constituents of the secondary envelope of the chorion [6]. The ZR also known as the zona pellucida, chorionic vitelline envelope, chorion or vitelline membrane, has been studied in several orders and species of fish [2-8,11-21]. The ZR covering the egg is generally a complex extracellular matrix with pore-canals filled by oocyte microvilli and follicular cell slender processes, and usually consists of two main layers with different morphological characteristics [21]. The inner layer consists mainly of proteins with few carbohydrates, and is similar to the zona pellucida of mammals. The functions of this layer in fertilization and embryonic development have been conserved during evolution. The outer layer has a specific macromolecular composition containing glycoproteins, carboxylated and sulfated polysaccharides and, rarely, sialic acid, all of which contribute to the interactions between the egg and its aquatic environment [21]. Ultrastructural analysis has shown that the ZR is formed during the previtellogenic stage [2].

In this paper, the growth of the ZR during the late stages of oocyte development was studied in *Liza aurata*, a marine teleost fish species, and the findings were compared with reported data in the literature.

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MATERIAL AND METHODS

Sample collection

Females of *L. aurata* were collected from catches made with beach seines along the Anzali shore (Guilan province, Iran) in late September, which corresponds to the reproductive period of mullet. The developmental stage of the ovaries was determined using a special, very thin, hand-made, sampling canula and only fishes with ovaries in the stage IV of development were selected.

Induction of ovary development

Ovarian growth in *L. aurata* has been divided into six stages (the first five are oocyte growth stages and the 6th stage is a spent one). Since *L. aurata* avoids coastal waters during preparation for spawning, the late 4th and 5th staged ovary are rarely caught. Therefore, to obtain the desired ovarian stage, the fishes used were injected with a carp hypophysial extract (20-30 mg/kg, two injections at 12 h intervals).

Histological preparation

Samples of ovary fixed in Bouin's solution were processed and embedded in paraffin. Sections 6 μm thick were stained with hematoxylin-eosin for light microscopy. The thickness of the ZR was measured with an ocular micrometer in ZR randomly selected regions of at least 25 similarly staged oocytes and the mean value was calculated. The sections were photographed using an Olympus microscope and photographic system. All of the photomicrographs are shown as the actual magnification, except for figure 1C, which was magnified a further three fold to provide a clearer view of the pores.

RESULTS

All five stages of ovarian development were identified in the histological sections. The protoplasmic content of the oocytes in stages I and II (previtellogenic) was basophilic and the ZR, although already present, was not recognizable by light microscopy. In stage III (early vitellogenesis), a ZR about 11 μm thick with marginal striation was observed (Fig. 1A,B). Perforation of the ZR was identified by dotted appearance of its inner surface (Fig. 1Cc). In the stage IV of development, the oocytes increased in size, there was extensive yolk deposition which became acidophilic and the follicular and ZR layers were better organized. Bud–like structures were seen in the ZR and gradually developed into protrusions or primitive projections that were directed inwards; into the perivitelline space (Fig. 2A,B,C). The striations were still seen along the primitive projections and were about 28 μm long. A perivitelline space was present between the oolemma and the ZR (Fig. 2B,C). In late vitellogenesis (late stage IV), the primitive projections extended into finger-like structures about 45 μm long (Fig. 3A) that penetrated into the perivitelline space (Fig. 3B). The number of these structures toward perivitelline space increased progressively along with oocyte further development. Many pores were recognized on the finger-like structures (Fig. 3C). Stage V consisted of a homogenous yolk, one or two large vacuoles, and loose layers of the follicular cells and ZR. The ZR was greatly reduced in thickness when vitellogenesis was completed in stage V (Fig. 3D).

DISCUSSION

Ovarian developmental stages in *L. aurata* consisted of immature (I), early mature (II), late mature (III), mature (IV), ripe (V) and spent (VI) stages [22]. Yolk deposition (vitellogenesis) in the form of globules, which began at the end of the stage III, continued until the onset of the stage V. At this stage, the yolk globules became homogenous and the yolk was eventually distributed. The onset of vitellogenesis (stage III) coincided with vacuolation of the ooplasm, with small vacuoles gradually uniting to form larger ones during stages III and IV. These vacuoles eventually fused to form one or two very large vacuoles in stage V [12]. In intact oocytes, the small vacuoles contained oil droplets and progressive fusion of vacuoles formed large oil drops which enabled the fertilized eggs to elevate from deep spawning ground and also to float on the water surface [12].

Light microscopy showed that the ZR was striated in early vittellogenesis. A similar finding has been reported for *Hemiodus* spp. [3]. Garcia-Diaz *et al.* [9,10] found such striations during late vitellogenesis in *Serranus cabrilla* and *S. atricauda.* In the ZR of *L. aurata*, each striated line represented a canal with pores opening at both ends (Fig. 1C). Abdalla and Cruz-Landim [1] also observed a perforated ZR with pore canals in *Piaractus mesopotamicus*.

The ZR canals seen in *L. aurata* during vitellogenesis may be involved in the transfer of vitelline contents to the oocyte. Circulating vitellogenin reaches the oocyte via the pore canals of the ZR being receptor-mediated endocytosed into the ooplasm [8]. Brandão *et al*. [3] suggested that the existence of such pores, in adhesive and non-adhesive eggs was related to metabolic changes (oxygenation) in the oocyte after spawning. Cruz-Höfling and Cruz-Landim [6] stated that the chorionic fibrils (CF) of *Crenicichla johanna* (Cichlidae) had axial holes that could serve as a very thin capillary system for gas exchange in water.

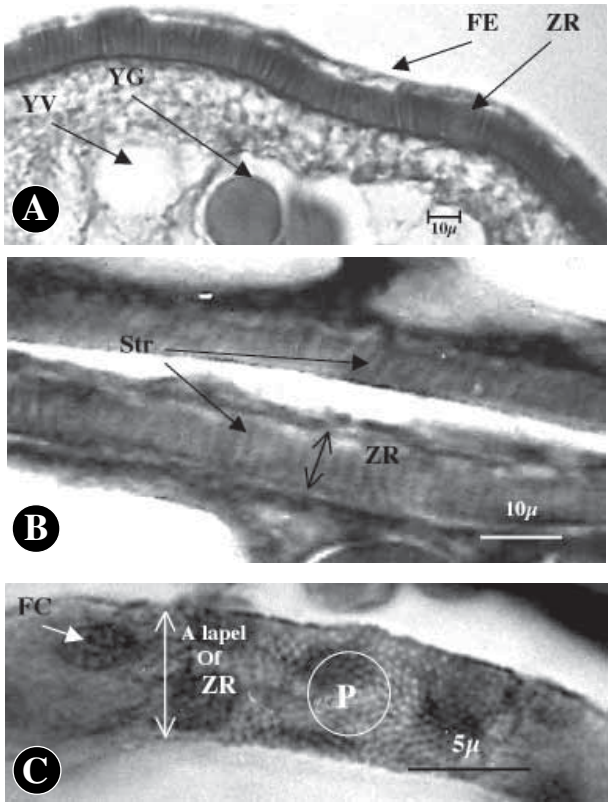

Figure 1. The zona radiata (**ZR**) of an *L. aurata* oocyte in early vitellogenesis (stage III). **A**) ZR with striations and a follicular layer. **B**) Striated ZR of two adjacent cells. **C**) A lapel of ZR showing dot-like pores. **FE**-follicular epithelium, **P** – pore, **Str** – striation, **FC** – follicular cell, **YG** – Yolk globule, **YV** – Yolk vesicle.

As shown here, during further growth of the oocyte and vitellogenesis (transition from stage III to IV), the ZR in *L. aurata* played an increasing role in the transport of material required for yolk synthesis. The porous, finger–like projections reached a length of 45-50 μm at the end of the 4th stage. These structural changes in the ZR were correlated with the appearance of bud-like, primitive projections that later extended as finger-like structures. This structural change resulted in an increase in surface area and agreed with the importance of the ZR in transporting the material necessary for production yolk during vitellogenesis. Elongation of the ZR projections, which was accompanied by an increase in the number of pores during this period (IV stage), enhanced the entry of raw material through pores involved in vitelline production and hydration. Whereas the transition from stage III to stage V in *L. aurata* normally requires 10-15 days, the injection of a carp pituitary extract hastened this process to 24-36 h [12,22]. This thickening resulted from the deposition of new layers of ZR (chorion) during oocyte ripening. Such a marked thickening during the development of porous canals in ZR, with peak of vitellogenesis in late stage IV, has not been recorded before. In addition, a perivitelline space was noted between the ZR and oolemma, as also seen in *Crenicichla johanna* [6].

Since *L. aurata* spawns in offshore waters and the fertilized eggs are planktonic, there is no need for the eggs to adhere or anchor to any substratum. Hence, in *L. aurata* the ZR is not involved in anchoring oocytes, although the ZR is known to be adhesive [4,14,20,21]. Park and Kim [13] stated that the nature of the ZR in three-spined loaches, *Iksokimia logicorpus* (ZR with villi), *I. hugowolfeldi* (ZR with short thin villi) and *I. yongdokensis* (ZR with round granules) could specify the habitats of these fish. The first of these species inhabits pebble bottoms with

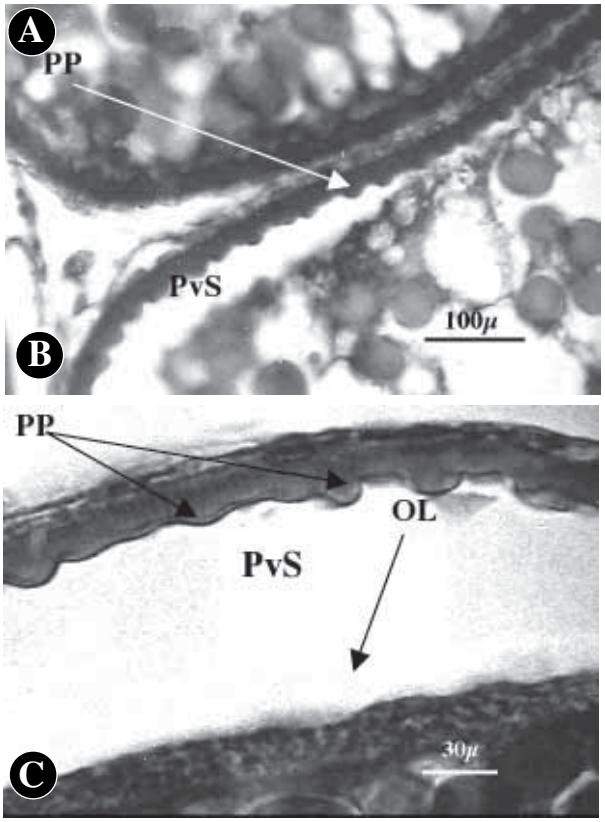

Figure 2. Oocyte during the vitellogenic period (early stage IV). **A**) Bud-like structures with the primitive projections. **B**) Primitive projections or protrusions with a previtelline space. **C**) Elongation of projections. The previtelline space have been formed between the ZR and oolemma, **ZR** – zona radiata, **PP** – primitive projections, **YG** – yolk globule, **YV** – yolk vesicle, **PvS** – previtelline space, **OL –** oolemma.

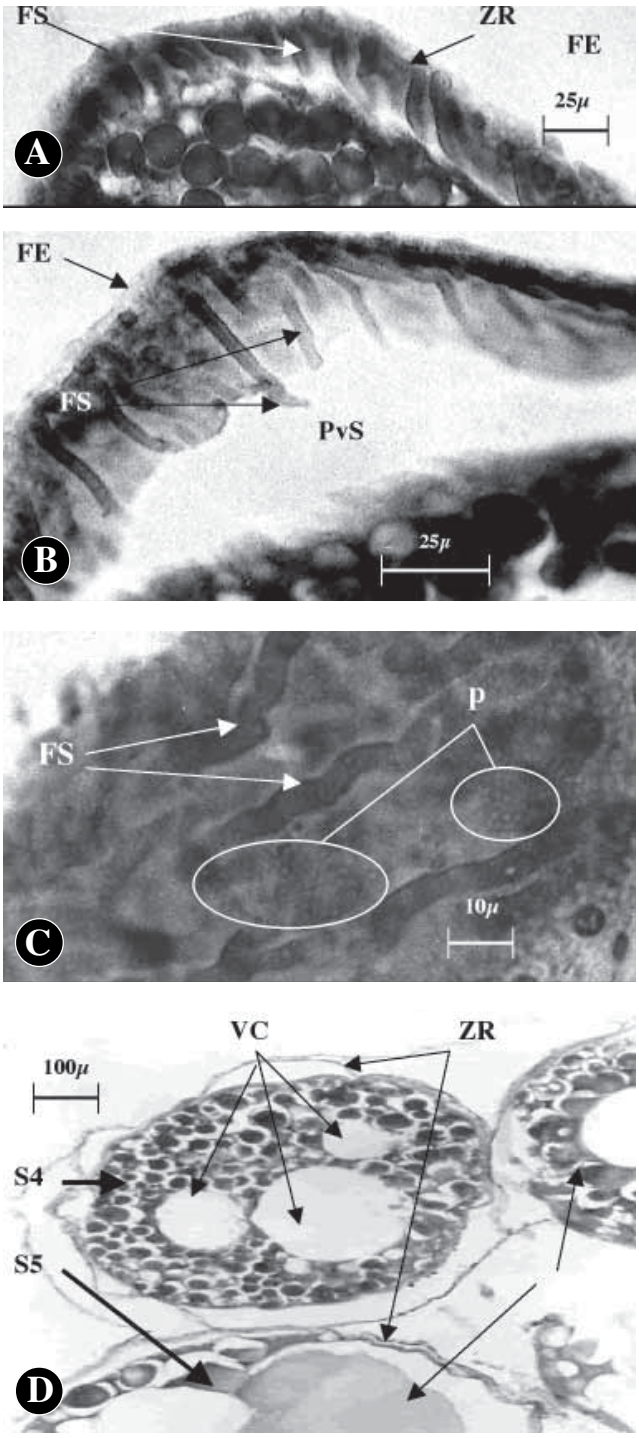

Figure 3. The ZR in late vitellogenesis (late stage IV). **A**) ZR showing finger-like structures. **B**) Finger-like structures extending to the previtelline space. **C**) Dot-like pores on the finger-like structures and also elsewhere. **D**) Course of transition to stage V. Fusion of yolk globules and vacuoles resulting in fewer vacuoles in S4 and a homogenous yolk in S5. The ZR is thin and loose around the oocyte. **FS** – Finger-like structure, **PvS** – perivitelline space, **FE** – follicular epithelium, **P** – pore, **ZR** – zona radiata, **Y** – yolk material, **VC** – vacuoles, **S4** – onset of stage V, **S5** – stage V oocyte with a homogenious yolk.

rapidly flowing water, the second inhabits pebble or sand bottoms with moderately flowing water and the third inhabits mainly sand bottoms with slow-flowing or stagnant water. Rizzo *et al.* [21] suggested that the presence of adhesive and non-adhesive eggs in characiform and siluriform fish was related to features of the ZR. In characiforms, adhesive eggs had a ZR with globules, filaments, villi or honey comb-like pores, whereas non-adhesive eggs of the same order, had a smooth ZR with pore canals or a fibrillar network on the surface. In weakly adhesive eggs, the ZR showed only pore canals. The jelly coat of siluriform eggs showed no adhesiveness [21].

Chorionic fibrils may help in attaching of the eggs to a substratum and in preventing water loss during low tides. When the eggs are exposed to air, the fibrils may function as a respiratory system [6].

The eggs of *L. aurata* are pelagic, buoyant and have a small diameter compared with demersal eggs. The chorionic structure reflects adaptations to variable environmental conditions. Pelagic eggs usually have a thin chorion whereas demersal eggs have a thicker, more complex chorionic membrane [21]. Ivankov and Kurdyayeva [11] discussed the thickness of the ZR in relation to different reproductive strategies, and suggested that the ZR is thicker in eggs subjected to heavy mechanical stress, in contrast to floating eggs or those located in sheltered areas.

The floating eggs of *L. aurata* in stage V of ovarian development lost their organization seen in the previous stage, with their yolk content becoming homogenous and fully hydrated before spawning. The finger-like structures of the ZR disappeared and the thickness of the layer decreased to 27 μm. Similar physical changes in the ZR have been reported for *Serranus cabrilla* [10].

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