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COMPARATIVE STUDY ON THE STRUCTURE OF ZONARADIATA IN EGGS OF THE COMMON CARP (*CYPRINUSCARPIO*) AND THE GRASS CARP (*CTENOPHARYNGODONIDELLA*) FROM PRE-VITELLOGENIC TO POST-FERTILIZED OOCYTE

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ABSTRACT

Zonaradiata (ZR) is an area that originates in early stages of the oocyte growth coveringoolemma, grows and thickens gradually during developmental stages. The structure of the ZR provide exchange of nutritional material and gases during vitellogenic and embryonic stages meanwhile could be related to habitat and spawning location of the fishes. Light and scanning electron microscopic studies on the histological sections of ovaries and oocytes in both the common carp (*Cyprinuscarpio*) and the grass carp (*Ctenopharyngodonidella*) showed zonaradiataappearence in previtellogenic stage (cortical alveolar stage) as an amorphous and simple acellular layer that reached its maximum thickness and complexity at vitellogenic stage. In post-vitellogenic (mature) and fertilized oocytes, the zonaradiata of both species gained simplicity and reduced in thickness. Obvious difference in thickness, architecture and number of pore-canals were seen among ZR of all stages of oocyte development of both species. Also, in common carp mature eggs unlike to the grass carp eggs, a gelatinous or jelly coat was seen around the ZR. Such features could present species – environment ties and as remedies to reproduction obligations.

Keywords: Zonaradiata, Oocyte, Common Carp, Grass Carp

INTRODUCTION

Zonaradiata (ZR) or otherwise called zonapellucida [13], chorion[12], eggshell [28], egg membrane [33], egg envelope [5], vitelline membrane [9], external membrane [16] and vitelline envelope [34], is an area appearing between oocyte plasma membrane (oolemma) and follicular envelope [1]. The ZR changes gradually during ovarian developmental stages under management of oocyte and follicular cells both [26]. ZR main functions include transport of yolk material, egg fixation to the substratum, sperm attraction, prevention of polyspermy and antibacterial and mechanical protection during spawning, fertilization and post-fertilization periods [34]. For the developing embryo, the ZR enables gas exchange, excretion and transport of nutrients from the external environment [30], [34]. The ZR was suggested as a possible biomonitor for ecological contaminants [5] and biomarker for environmental estrogens [3].

There are very conflicts about the number of the layers of the ZR. Some researchers believe that the ZR in all species of the bony fishes is consisted of two layers, the thin zonaradiataexterna (ZRe) and the thicker zonaradiatainterna (ZRi) [29]. In seahorse (*Hippocampus erectus*), pipefish(*Syngnathusfuscus*) [1] and *Serrasalmusspilopleura*, the ZR has three layers named Z_1 (the oldest layer), Z_2 (middle layer) and Z_3 (the youngest layer), from outer to inner side respectively. [7]demonstrated that the egg envelope ofthe white sturgeon (*Acipensertransmontanus)* was consisted of four layers, L_1-L_4 including the outermost jelly layer. On the other hand, some researchers do not account L_4 (jelly layer) as a layer of the ZR, because it is not secreted before the activation of eggs [8]. In some bony fish species such as the viviparous fishes, for example, *Xiphophoroushelleri*[26], the ZR is simple and non-layered. Comparative ultrastructural studies of the ZR of marine fish eggs in three genera (four species) of Perciformesby Li (2000) [24] demonstrated that there have been differences on distribution density of pores on outer surface of the ZR among species. The ZR surface and distribution density of pores are useful characters for distinguishing among the four species. Based on ultrastructure of the ZR in the common carp (*Cyprinuscarpio*) Shabanipour and Hossayni (2010) [32] also established information on density, size and connection of pores on both outer and inner surfaces of ZR.

For the present study, two cyprinid fish- the common carp (*Cyprinuscarpio*, Linnaeus 1758) and grass or white carp (*Ctenopharyngodonidella*, Valenciennes 1844) were selected and the growing ZR during oocytes developmental stages and their post-fertilization modifications were evaluated. The selected species belong to separate genera and differ from each other in various aspects of biology such as feeding habits, living environment, breeding ground and strategies. Because of different level of systematic position difference in fine structure of the ZR would be expected particularly at pre- and post- fertilization stages of eggs.

MATERIALS AND METHODS

Sample collection

Samples (ovaries, mature and fertilized eggs) of the common carp and the grass carp were obtained from local private fish farm.

Histology

All samples were fixed in Bouin's solution for 24 hours and then dehydrated in a series of graded methanol, cleared in xylene, embedded in paraffin wax and sectioned at 5 µm thickness by rotary microtome. Sections were stained by hematoxylin and eosin stains and mounted permanently.

Microscopy

Tissue sections were studied under light microscope (Olympus), photomicrographs provided and evaluated by TSView camera and software. To exploit the details of events happening in stained prepared slides, a single desired unstained paraffin section was selected out of a known serially sectioned ovary tissue or oocyte and glued on a small 1 cm × 1 cm cover slip. The section was then deparaffinized by xylene and cleared by 90% methanol for several times [20]. In the SEM Center at the University of MohaghеghArdabili, cover slips were mounted on stubs, gold coated, scanned by LEO 1430VP SEM and measurements were made by LEO serv-32 software.

RESULTS

Histological preparation as well as SEM micrographs of primary growth stage did not showed any sign of ZR.At the previtellogenic stage (cortical alveolar stage), the zonaradiata (ZR) was formed by growing of outgrowths (microvilli) from the oocyte and follicular cells surface toward each other and deposition of substances around the microvilli (Fig. 1B). At this stage, the ZR seemed as a thin, simple and acellular layer between the oocyte plasma membrane (oolemma) and follicular envelope (average thickness in common carp and grass carp were 0.75 and 0.4 micron, respectively).

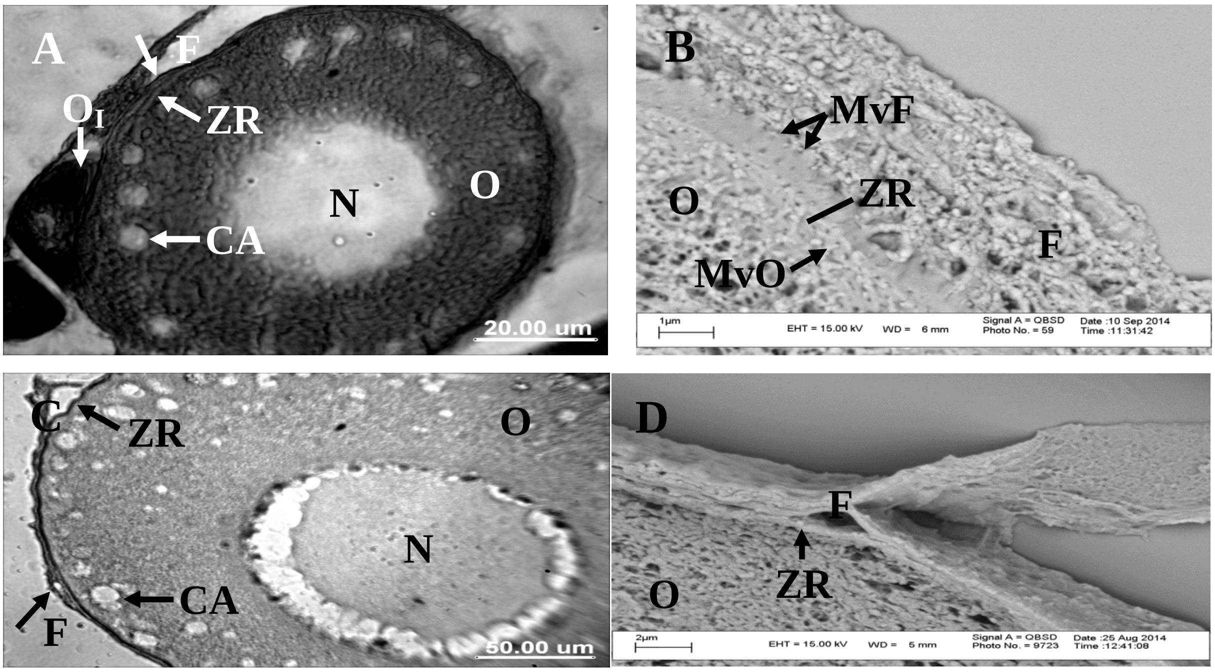

Figure 1. Stage II oocytes; A- and C- Photomicrographs of the common carp and the grass carp, respectively; B- and D- SEM micrographs of the common carp and grass carp, respectively, ZR: zonaradiata, CA: cortical alveoli (yolk vesicles), N: nucleus, O: ooplasm, F: follicular envelope, O_I: oocyte in primary growth stag (stage I), MvF: microvilli from follicular cells, MvO: microvilli from oocyte surface.

In early vitellogenic oocytes (stage III) (Fig. 2A), the ZR attained further thicknessand complexity grade. Pore-canal organization became obvious. It is appeared that microvilli penetrated the ZR material and made canals from inner surface (oolemma side) to outer surface (follicular epithelial side). In late vitellogenic stage oocytes, the ZR reached maximum thickness in both species (average thickness in common and grass carps were 8.4 and 2.8 micron, respectively) and complications (Figs. 2B to E). Follicular envelope was present around the oocytes of both species (Figs. 2A to E). Fence-like striations (stria) or canals were clearly distinguished in histological preparations (Figs. 2B and D) of the ZR and microvilli were observedpenetrating into canals (Figs. 2A, C and E). Yolk granules in both species and lipid droplets or globules in grass carp occupied entireooplasm (Figs. 2B and 2D).

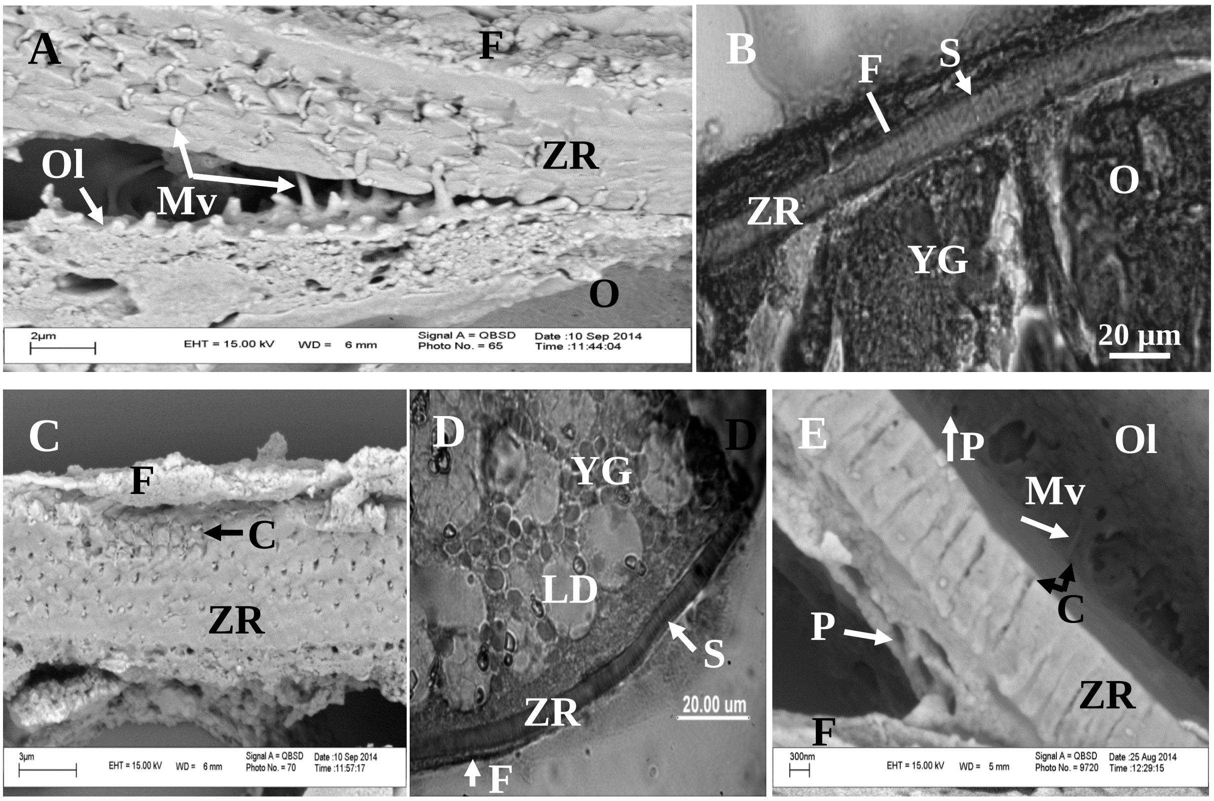

Figure 2.Vitellogenic stage; A- and C- SEM micrographs of ZR in common carp; B- and D- histological photomicroghraphs of oocyte coverings in common carp and grass carp, respectively; E- SEM micrograph of ZR in grass carp; ZR: zonaradiata, F: follicular envelope, O: ooplasm, Ol: oolemma, YG: yolk granules, LD: lipid droplets or globules, S: striations (stria), C: canal, P: pore, Mv: microvilli.

In post-vitellogenic (mature) oocytes (stage IV), the thickness and the complexity of the ZR reduced apparently in both fish (Fig. 3) (average thickness in common carp and grass carp were 5.5 and 2.3 micron, respectively). Yolk granules joined each otherresulting into formation of yolk plates(Figs. 3A and 3D). In both species, the ZR were spaced in a distance from the oolemma and a wide space was formed between them called perivitelline space (Figs. 3A and 3D). Large evaginationsalong cell margin were produced in the ZR of the grass carp giving it wrinkled appearance (Fig. 3D). Most canals were occluded or disappeared and traces were present as shallow grooves. Microvilli were not observedanymore. A potential gelatinous or jelly coat was seen around the ZR of the common carp eggs (Fig. 3A). The ZR in common carp posses fewer pores on the inner surface but larger in size and did not show a specific arrangement(Fig. 3B), The ZR outer surface though exhibited to be morein number, smaller in size and arranged linearly (Figs. 3C).

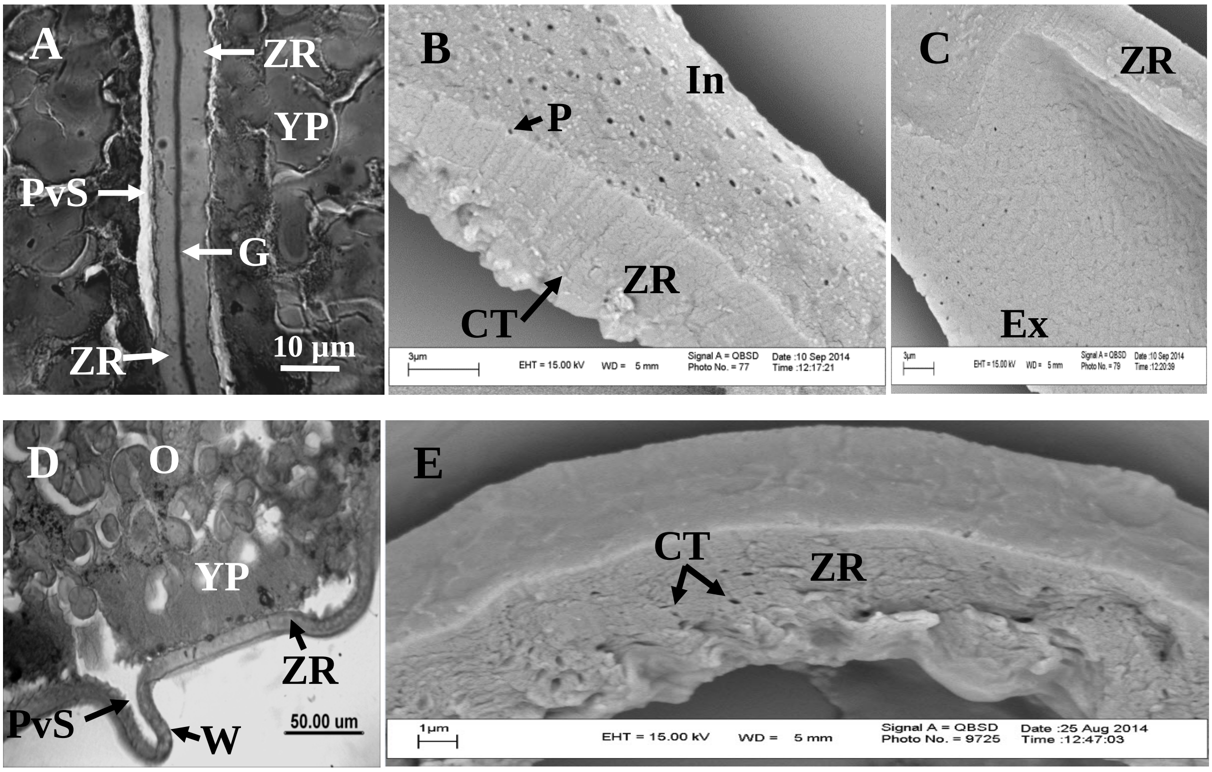

Figure 3. Post-vitellogenic (mature) oocytes; A- and D- photomicrographs of the common carp and grass carp, respectively; B- and C- SEM micrographs of the common carp(when gelatinous coat was removed) and E- SEM micrograph of the grass carp; ZR: zonaradiata, G: gelatinous or jelly coat (of two adjacent oocytes), CT: canal trace, Ex: external surface of the ZR, In: internal surface of the ZR, P: pore on the opening of canal, YP: yolk plates, O: ooplasm, PvS: perivitelline space, W: wrinkle.

POST - FERTILIZATION FEATURES

Further reduction in depth and number of pore-canals (striations) continued in both fishes (the average thickness of the ZR in common carp and grass carp reached 2.2 and 1.05 micron, respectively) (Fig. 4A and B, 4C to E). Pores and canals were almost disappeared in grass carp ZR covering oocyte and seemed as a compact and homogenate structure approximately similar to its initial form (Fig. 4C to E). Certain traces of canals were seen in the common carp eggs (Fig. 4B and C). The perivitellineformed wider space and the ZR was elevated with a distance from thickened oolemma region (Figs. 4A, C and D). Inthis stage, the modified ZR is called "fertilization envelope or membrane". The number and length of the wrinkles were increased in grass carp eggs (Figs. 4C and D).

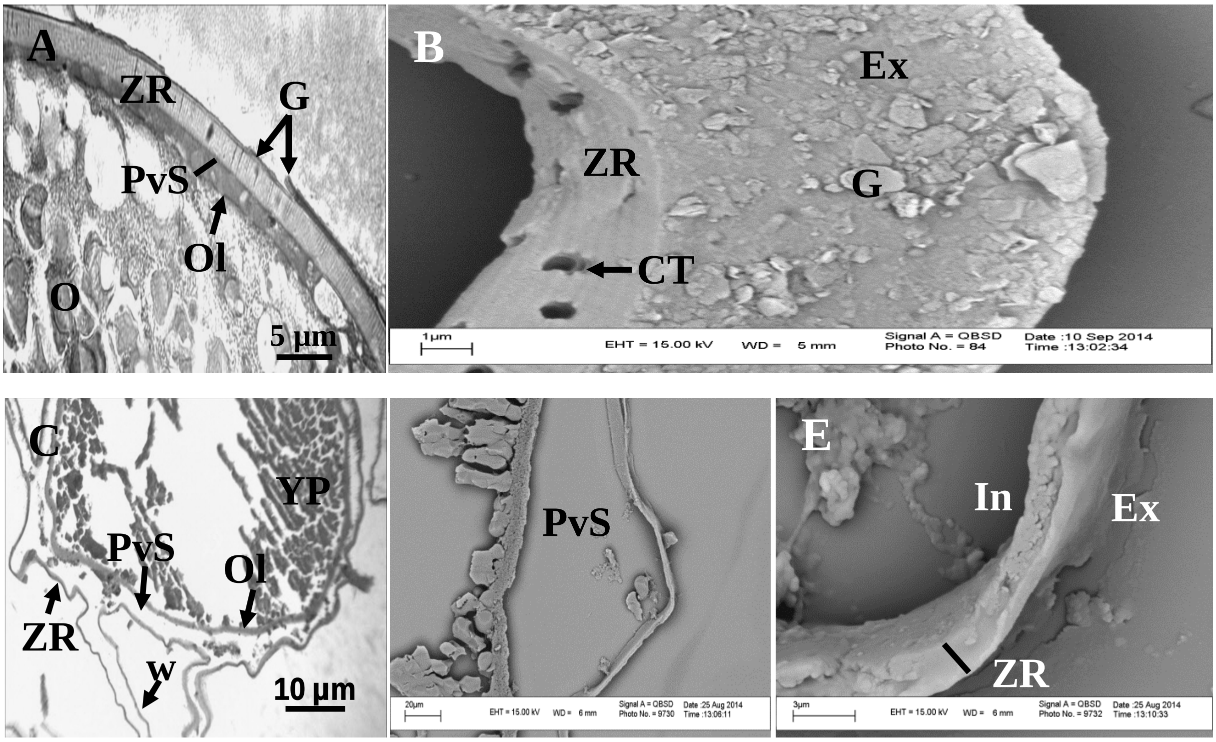

Figure4. Fertilized oocytes; A- and C- Photomicrographs of the common carp and grass carp, respectively; B- SEM micrograph of the common carp and D- and E- SEM micrographs of the grass carp; ZR: zonaradiata,G: gelatinous or jelly coat, In: internal surface of the ZR, Ex: external surface of the ZR, PvS: perivitelline space, Ol: oolemma, O: ooplasm, W: wrinkle, YP: yolk plate, CT: canal trace.

DISCUSSION

In both carp species, the ZR did not appear at previtellogenic stage I (primary growth stage), though in *Pseudosciaena crocea* it was suggested to be initiated at this stage. At the present study, the ZR was first observed at previtellogenic stage II (cortical alveolar stage) as a thin, simple and acellular layer around the oocyte, which increased gradually in thickness and structural complexity during further development of oocyte passing from the cortical alveolar stage to the vitellogenic stage. Other researchers have also reported the origination of the ZR at cortical alveolar stage [1], [20], [23], [31], [32]. Despite the agreement of researchers on the origination of the ZR in previtellogenic stage, Koc (2012) [22] reported the appearance of the ZR of the zebrafish eggs in vitellogenic stage.

During vitellogenic stage (stage III), the ZR gradually attained maximum thickness and complexity grade of pore-canals organization (Fig. 2). Vitellogenesis has been denoted as a very complex process that need active transport of raw materials from blood to oocytes [6], therefore the gradual increment and overall better defined organization of the ZR, exhibited an effective role to be performed as e route for transport of essential materials to be incorporated in yolk synthesis (during vitellogenesis).

On oocyte sections, the striation of the ZR are actually the canals or passageways made by microvilli or processes originating from oolemma and follicular cells towards each other[26]. The canals were presented in different size and do not go straight forward along the ZR (Figs, 2A, C, E). All canals open to the surface directly or indirectly by means of a pore on either sides of the ZR layer (internal or external). In the common carp the pores on internal and external surfaces of the ZR (Figs. 3B and C) differ in number and size. But how this inequality in pore-canals and also direction of pathway of canals act in transportation of materials, is yet unknown. These properties may demonstrate a type of selective transport [26].

In post-vitellogenic (mature) oocytes, the ZR found quite simple architecture and its complexity (fence-like striations) was reduced tremendously (Fig. 3A, D and E). In commom carp it was appeared that the canals were occluded andinner surface pores though larger in size were few in number compared with smaller and more pores on outer surface.In grass carp on both inner and outer surfaces of ZR the reduction in number of pores was impressive in such a way that pores were rarely observed.These results confirm the findings of Hurley and Fisher (1966) [16] which demonstrated that when the rainbow trout eggs maturated, the number of pore-canals reduced because of their occlusion. These changes may exhibit the reduction of nutritional and increment of protective role of the ZR (particularly in the grass carp eggs).

After fertilization, Outside the ZR in zygotes of the common carp, a gelatinous or jelly coat was appeared. Common carp lives and spawns in warm and slow waters and this envelope can act for adhesion of eggs to the spawning substrate (macrophytes) [25]. The absence of such covering over the fertilized eggs of grass carp is believed to be related to the spawning habitat of this fish. Grass carp lives and spawns in fast waters. The eggs are semipelagic, therefore they were not provided any device or material for attachment to substratum[21]. It might be argued that the wide perivitelline space and wrinkles of ZRwould protect the eggs from the high mechanical pressure of the external environment. The studies of Huysentruyt and Adriaens (2005) [17] on the catfish (*Corydorasaeneus*) and Heidari et al. (2009) [15] on the cyprinid fish Kutum (*Rutilusfrisiikutum*) revealed that generally in teleosts, small protuberances, attachingfilaments or microvilli-like adhesive structures perform such roles. In fertilized eggs, the reduction in thickness and structural complexity of the ZR was further proceeded (Fig. 4). The striated feature of the ZR was approximately lost and the ZR changed to a nearly homogenated and elevated region. Perivitelline space (the space between oolemma and the ZR) became wider and oolemma region showed to be thicker (Figs. 4A, C and D). It is appeared that these changes are the result of cortical reaction, *i.e.*, at the time of fertilization, cortical alveoli attach to the oolemma and exit their contents into perivitelline space by exocytosis and change the structure of the ZR [14], [19]. Thus, this area is hardened and elevated and transformed to the fertilization envelope or membrane. The histochemical composition of the CA, which also varies among species, is made up of glycoprotein and/or glycoconjugate[4]. Transformation of the ZR by the alveolin and transglutaminase with reduction in diameter closes the micropyle and blocks polyspermy[27]. Iwamatsu (1983) [18] showed that when oocytes were dechorionated (the ZR removed or separated) and massaged with sperms, polyspermyoccured.

We saw that differences in ZR fine- and ultra-structure between two species are remarkable. For example, the number of layers, architecture of pore-canals and existence or absence of microvilli were different. This is to be expected, because these two species belong to different genera (*Cyprinus* and *Ctenopharyngodon*), as comparative ultrastructural studies of the ZR of marine fish eggs in three genera in perciformes revealed that the studied characters (absence or presence and distribution density of pores) did not differ significantly for the fishes in the same genus, but was significantly different for different genera, even when the genera were in the same family [24].

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