Histomorphological investigation of *Liza aurata* (Risso, 1810) (Mugilidae) ovary in the late oogenesis in the Caspian Sea

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Received: November 2007 Accepted: November 2008

Abstract

In the present study, various developmental stages of *Liza aurata* oocyte, especially IV and V stages have been described. On the basis of histological investigations, oocyte development in *L. aurata* comprises immature (I), the early maturing (II), the late maturing (III), mature (IV), ripe (V), and spent (VI) stages. In the stages I and II, nucleus occupied large volume of oocyte. Vacuolization and vitellogenesis appearance started at stage III. Vitellogenesis increased by further growth of oocyte at stage IV and also vacuolization occurred. Zona radiata and follicular cells were more conspicuous at this stage. In the late stage IV, number of vacuoles decreased due to fusing of small vacuoles and nucleoli located on different places of nucleus at this stage. At stage V, oocyte normally possessed one or two oil droplets; nucleus disappeared after migration to animal pole. Recently spawned oocytes were fluid, lemon in color and 779.2μm in diameter. The maximum gonadosomatic index (GSI) value was found at stage V.

Keywords: Oocyte, Ovary, Vitellogenesis, *Liza aurata*

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Introduction

Liza aurata (golden grey mullet) and *Liza saliens* were introduced to the Caspian Sea from the Black Sea in 1930-1934, acquiring good acclimatization in the new environment. Nowadays, *L. aurata* constitutes an important commercial catch along the Iranian coast of the Caspian Sea. The members of this family (Mugilidae) are coastal marine and less frequently freshwater fishes of wide distribution in tropical and subtropical waters (Fazli *et al.*, 2008). The reproductive strategy (synchronous or asynchronous development of oocytes) indicated how oocytes sexually mature and ovulate. Recently, efforts have been forwarded to manipulate total or partial sagametogenesis in species such as salmon, mullet, and milkfish by hormontherapy. Ovary tissue contains main components namely oocytes, germinal layers, connective tissue, stem cells and vessels. The oocyte consists of cytoplasm, nucleus, cell membrane and yolk globules in more developmental stages. Maturation is known as behavioral, morphological and physiological changes that currently occur with variations of gonads and levels of hormones. In fishes, the length of ovary increases and periodic changes occur in diameter of oocytes (Evans, 1993).

Most fishes have periodic reproductive behavior in which the study of maturation could be followed by examinations of related histology and morphology on ovaries (Evans, 1993). Kulikova and Loshakova (1982) had investigated oogenesis and sexual cycle of the *L. aurata* in 1982. In addition, Hotos *et al.* (2000) had studied the reproductive biology of *L. aurata* in the lagoon of Klisova (Greece) in which they could not acquired final (ripe) stage of maturation. Investigations on the ovary of several marine and freshwater species have been studied (Garcia-Diaz *et al.*, 1997; Andrade *et al.*, 2001; Prisco *et al.*, 2001; Garcia-Diaz *et al.*, 2002; Munoz *et al.*, 2002; Ravaglia & Maggese, 2002; Rizzo *et al.*, 2002; Abdalla & Cruz- Landim, 2003; Brandao *et al.*, 2003; Corriero *et al.*, 2003; Grassiotto & Guimaraes, 2003; Duperly & Martha, 2004).

In the present study, progressive growth of oocyte in the late developmental stages of ovary were studied and compared with reported data.

Materials and methods

In the late September, which corresponds with the last stages of gonad development, female *L. aurata* were collected from commercially trapped specimens by beach seines in Anzali (Guilan province, Iran) and immediately transferred to the laboratory. Since *L. aurata* avoids coastal waters during spawning period, the specimens in late stages of IV and V of ovary are rarely accessible. These stages were, therefore, induced on captive specimens with carp pituitary extract (20-30mg/kg body weight, two injections at 12h intervals). Determination of ovary

developmental stages was achieved by a hand made canula with diameter of 2mm suiting the genital pore.

Stereomicroscope was used for morphological observations in which freshly oocytes were studied and photographed. For histological consideration, small fragments of the ovaries from the middle region were fixed in Bouin's solution (12h), dehydrated in a graded series of ethanol, and embedded in paraffin, then sectioned in 6μm thicknesses by a rotary microtome. Sections were stained by Haemotoxylin-eosin general staining method (Oliveira & Santos, 2004). The mean diameter of fifty oocyte, nucleus, zona radiata (surrounding layer of oocyte membrane) were measured randomly in the late developmental stages by ocular micrometer. The gonadosomatic index (GSI) was calculated using the following formula (Shabanipour & Heidari, 2004):

GSI = gonad weight / whole body weight × 100

Results

The features of six developmental stages are summarized in Table 1.

Stage I: Immature

Immature ovary was characterized by ovarian lamellae that contained immature oocytes. As the oocyte grows, the nucleus is enlarged to form the germinal vesicle that occupies main part of the oocyte (Fig. 1a). Nucleoli was distributed in the periphery of nucleus close to nuclear membrane (Fig. 1a). Ooplasm was observed intensively basophilic and navy blue in color.

Stage II: Early maturing

The size of oocytes increased with growth of ovary. Compare to stage I, cytoplasm was stained less with haemotoxylin. The number of nucleoli increased. Nucleoli was located on the membrane of nucleus (Fig. 1b).

Stage III: Late maturing (cortical alveolus stage)

The oocytes at this stage were in the process of vacuolization (cortical alveolar system) and primary yolk production. Cortical alveoli were initially appeared circumferentially in cytoplasm. In comparison with peripheral vacuoles, there were the larger vacuoles around the nucleus existed (Fig. 1c). Zona radiata was first observed as a thin layer between the oocyte and follicular cells. The presence of yolk granules, which turns as yolk globules, is described in these oocytes.

Stage IV: Maturity (vitellogenesis)

Morphology

The Oocytes could be observed by naked eye, were deep yellow in color. By further growth, the diameter of vacuoles increased but their numbers decreased at the late stage IV (Fig. 2a). The diameter of oocytes were 500-600μm (mean 561.5μm) in the early stage IV and 600-800μm in later stage (mean 681μm).

Histology

By completion of vacuolization, oocytes entered the stage of maturity. Vitellogenesis elevated at this stage and reached maximum at the end of stage IV. The enlargement of the oocyte during vitellogenesis was largely due to accumulation of yolk protein precursors (Fig. 3a). Accumulation of yolk globules and enlargement of vacuoles pressurized the nucleus. Therefore, the nuclear membrane became scalloped and, with further growth, it appears folded (Fig. 3b). Nucleus was 100-150 µm in diameter. The number of nucleoli increased (about 18-20) and distributed on different positions of nucleus (Fig. 3b). The follicular layer was prominent and zona radiata continued to enlarge, acquiring an elaborate structure (Fig. 3c). The diameter of zona radiata at different developmental stages of oocyte is brought in the Table 2. The germinal vesicle (nucleus) was progressively displaced toward the animal pole as yolk was accumulated centripetally within the oocyte (Fig. 3d).

Stage V: Ripe

Morphology

The ovaries were highly enlarged in which occupied the body cavity to a great extend. Oocytes were distinct, fluid and lemonish. Vacuoles containing lipid fused together and produced one or two ones (Fig. 2b). The diameter of oil droplet and oocyte were 320-370µm (mean 340µm), 650-800µm (mean 779.2µm), respectively.

Histology

At the beginning of this stage, the oocyte had a large germinal vesicle located halfway between the centre and periphery. The envelope of the germinal vesicle broke down (GVBD) in the animal pole. After GVBD, oocyte continued to enlarge by hydration. During ripening, the oocyte became more translucent as lipid and protein yolk droplets coalesce to form one or two vacuoles (Fig. 4a). Follicular cells were not recognized, but zona radiata was observed.

Stage VI: Spent

A freshly spawned ovary was full of empty follicles with a few intermittent atretic oocytes. The ovarian wall became thicker, unovulated oocytes often located in the stage III and IV (Fig. 4b). Wide gaps were observed in between the ovigerous lamellae as they had became shrunken after ovulation (Fig. 4b).

Gonadosomatic index (GSI)

The values of GSI were given in Table 3 at various developmental stages of ovary. The GSI value was very low during the immature stages and gradually increased until stage III. It rose steeply through stage IV attaining the peak in stage V followed by spawning of oocytes.

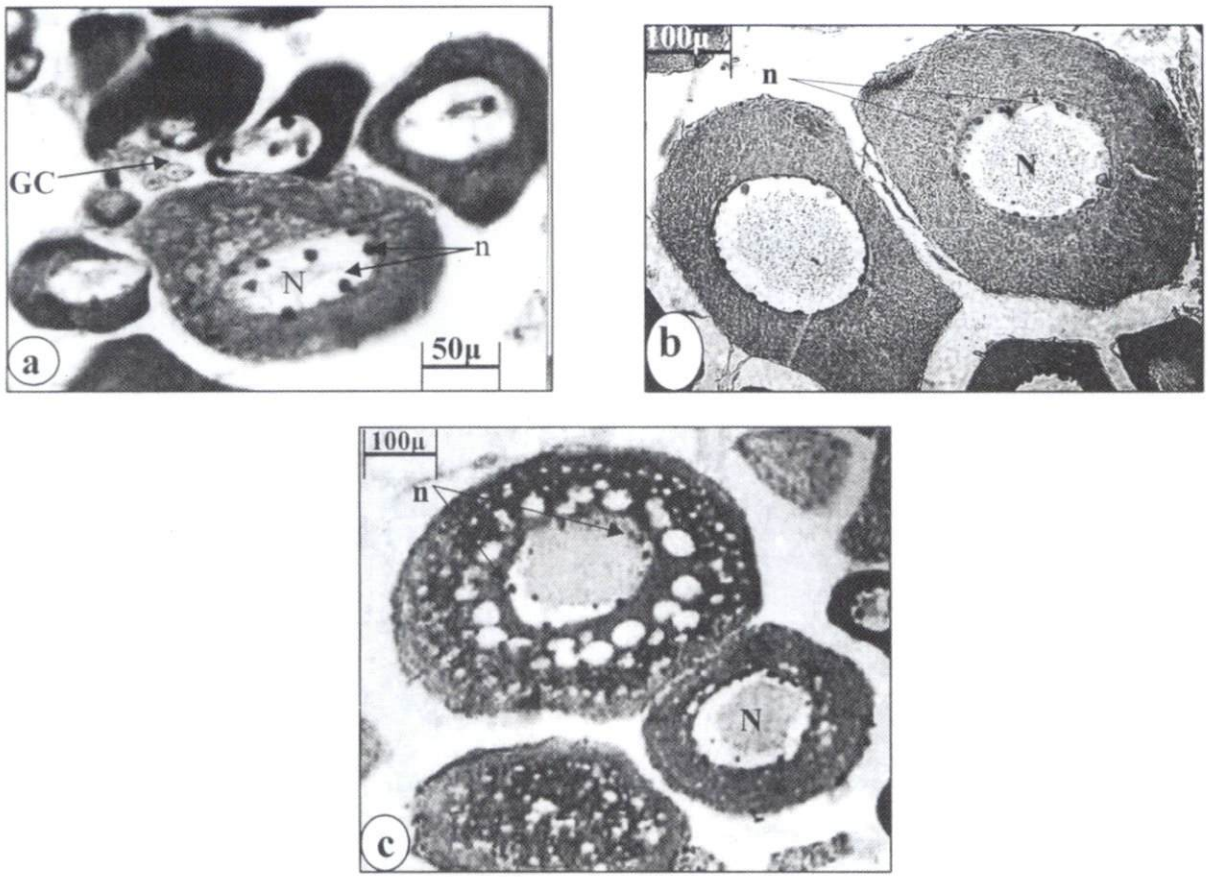

Figure 1: Histological view of immature stage or stage I, to the late maturing stage or stage III of *L. aurata* oocyte; a) photomicrograph of immature oocytes. Note the round and polygonal shape of oocyte, large nuclei and peripheral nucleoli (×100); b) photomicrograph of an early maturing oocyte (×100); c) section of ovary exhibiting late maturing oocytes wherein larger vacuoles were seen around the nucleus while the smaller ones were along the periphery (×100). GC: Germinal Cells; N: Nucleus; n: nucleolus.

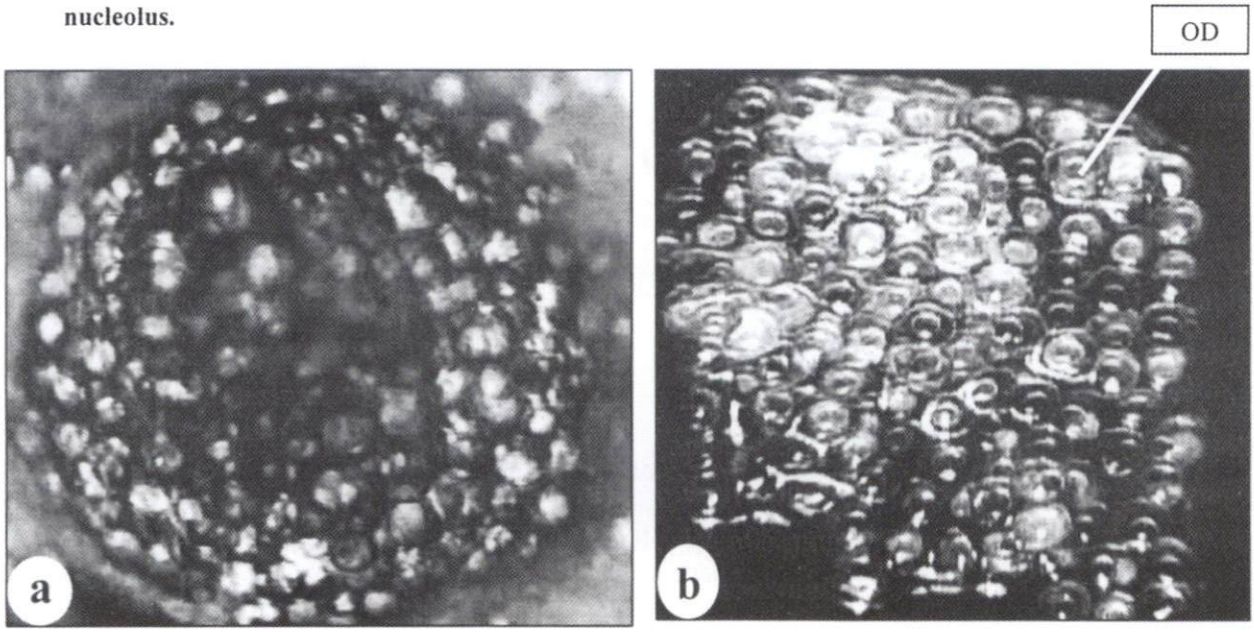

Figure 2: Morphology of oocytes at stage IV (a) and stage V (b). Note the oil droplet (OD) in the center of oocyte.

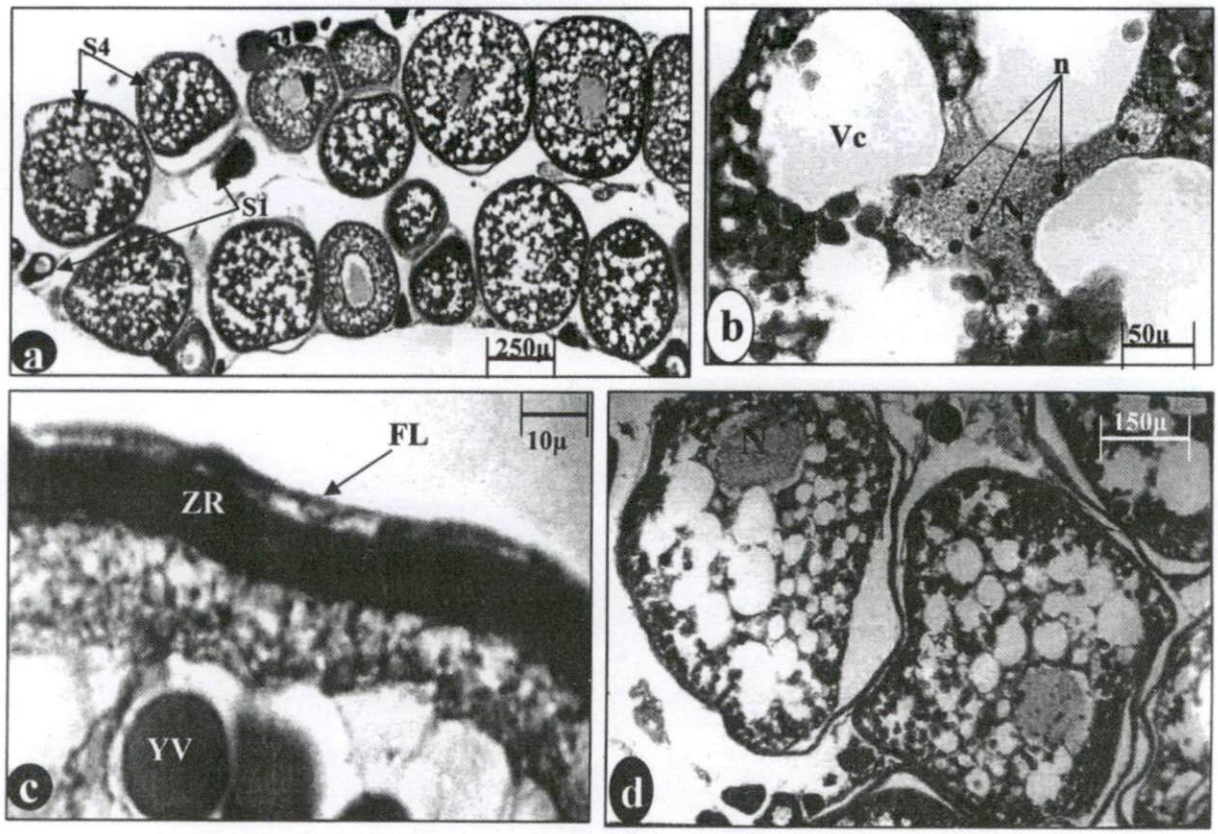

Figure 3: Histological view of mature (IV) stages of oocyte; a) enlarged view of mature oocytes showing acidophilic and fully vacuolated ooplasm (×40); b) nucleus of a mature oocyte with scalloped edges. Note the nucleoli distributed on different sites of nucleus (×400); c) enlarged view of follicular layer and zona radiata around the mature oocytes (×400); d) commencement of migration of nucleus of the mature oocyte to animal pole at the late IV stage. Note the nuclei distributed on different sites of nucleus (×100); FL: Follicular layer; N: Nucleus; n: nucleolus; S1: immature (I) Stage; S4: mature (IV) Stage; Vc: Vacuole; YV: Yolk Vesicle; ZR: Zona Radiata.

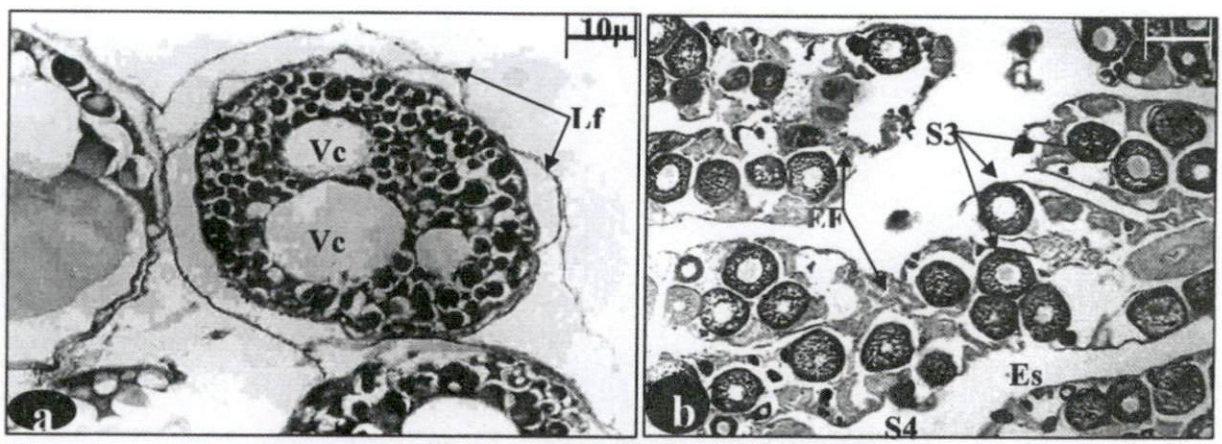

Figure 4: Section of ovary showing ripe a) and spent (b) ovary. Note homogenization of yolk, fusion of vacuoles and loosened follicular layer (×400); the spent ovary shows empty follicles with a few oocytes. Note shrunken ovigerous lamellae (×100); Es: Empty space between ovigerous lamellae; EF: Empty follicles; Lf: Loosened of follicular layer; Vc: Vacuoles; S3: late maturing (III) Stage; S4: mature (IV) Stage.

Table1: Classification of ovarian stages of *L. aurata* based on histological features

Maturity stages	Oocyte					
	Shape	**Ooplasm**	**Nucleus**	**Nucleolus**	**Yolk**	**Follicles**
Stage I (immature)	Round, oval, polygonal, small	Narrow, highly basophilic	Large and central	A few large ones, peripheral	Absent	Indistinct
Stage II (early maturing)	Oval, large	Broad, less basophilic	Nuclear membrane visible, chromatin material centrally placed	Number increased, size reduced	present	Distinct
Stages III (late maturing)	Round or oval, large	Commencement of vacuolization, primary yolk production	Round, definite nuclear membrane chromatin material central	Many in contact with nuclear membrane	Present	Prominent, possessing cubodial cells, zona radiata appears
Stage IV (mature)	Round or oval, larger	Numerous yolk globules, fully vacuolated	Scalloped, chromatin material visible, nuclear membrane indistinct	Number reduced, larger in size	Absent	Prominent, zona radiata well developed
Stage V (ripe)	Round or oval, largest	Homogenization of yolk, fusion of vacuoles	Migration to animal pole, distorted	Dispersed in ooplasm, insignificant	Absent	Thin and loose zona radiata present
Stage VI (spent)	Empty follicles with atretic and new crop of oocytes	—	—	—	—	Collapsed, empty follicle

Table 2: The thickness of zona radiata (ZR) in different maturity stages

Thickness of ZR (μm)	Maturity stage
Indistinct	Immature
Indistinct	Early maturing
11.6 ± 5.3	Late maturing
28.9 ± 6.48	Mature
44.5 ± 4.97	Ripe
27.5 ± 5.36	Spent

Table 3: The value of GSI in different maturity stages

Maturity stage	Months	GSI
Stage I (immature)	March	0.94±0.06
Stage II (early maturing)	June	1.35±0.18
Stages III (late maturing)	August	2.44±0.27
Stage IV (mature)	September	12.80±0.9
Stage V (ripe)	October	20.09±1.9
Stage VI (spent)	November	0.67±0.5

Discussion

The ovary of teleosts is cystovarian type except in Salmonidae and Notopteridae which is gymnovarian type (Hoar, 1969). A cystovarian type fish such as *Liza aurata*, encloses the oocytes by peritoneum or tunic, contrary to gymnovarian type which is naked and lack the peritoneum.

Observations showed that when the fish in stage IV were injected with hormone, more fusion of vacuoles and hydration

occurred leading to oocytes pass from stage IV to stage V.

Kulikova and Loshakova (1982) noted that oocyte sensitivity to pituitary gonadotropins originated concurrently with completion of their growth in the golden grey mullet while Rottmann *et al.* (1991) pointed out that hormones only were trigger for releasing of completely mature gametes. Histological studies on oocytes of *Mugil cephalus* revealed that oil droplets fused in response to hormone (Kulikova & Loshakova, 1982).

In *L. aurata*, stage III oocyte formed cortical alveolus system in which row of large and small vacuoles were arranged around nucleus and membranous cell, respectively. Merson *et al.* (2000) noted that cortical vacuoles first appeared in the vicinity of nucleus and then migrated to peripheral ooplasm near membranous cell. In contrary to this finding, it has been reported that first vacuolization of oocyte started in periphery of cell and progressed toward nucleus in the centre of oocyte (Caputo *et al.*, 2000; Ravaglia & Maggese, 2002). In many bony fishes commencement of vacuolization coincide with vitellogenesis, however in few fishes such as zebra fish (*Brachydanio rerio*) this may not be so (Merson *et al.*, 2000).

In golden grey mullet at stage IV, larger vacuoles containing oil were resulted from fusion of small vacuoles. At stage V (spawning time), only 1-2 vacuoles or oil droplets were seen (Figs. 3b, 4a). The diameter of oocyte and oil droplet was 650-800μm (mean 799.2μm) and 320-370μm (mean 340μm), respectively. Kulikova & Loshakova (1982) had reported 740-820 and 310-325μm, for diameter of oocyte and oil droplet, respectively. Because of fertilized eggs of mullet ascended to epipelagic waters from deep waters (300-700m depth), it seemed that oil droplets were utilized for buoyancy and ascending to the surface waters (Kulikova & Loshakova, 1982).

The extensive hydration made sudden increment in diameter of *L. aurata* oocyte in the late stages of maturation. Injection of hormone into *M. cephalus* resulted in increased volume of water within oocyte from 59.4% to 84.8% (Watanabe & Kuo, 1986). Ravaglia & Maggese (2002) assumed vitellogenesis responsible for sudden increment of oocyte volume till 90%. The increase in oocyte volume leads to rupture thin ovary wall at final stage.

Nucleus and nucleoli of *L. aurata* oocytes increased in number till stage IV where vitellogenesis reached to the maximum. Appeared changes in nucleus and nucleoli are attributed to synthetic active processes of yolk during ovary growth in different species (Kuo *et al.*, 1974; Brackeveltand & McMillan, 1967; Kulikova & Loshakova, 1982). Nucleoli were location for synthetic actions of nucleus and production site of ribosomal RNA (Nagahama *et al.*, 1993). It seemed that increase in the number of nucleoli directly related to vitellogenesis, that is the

number of nucleoli were maximum (18-20) during vitellogenesis.

Acknowledgment

This study was supported by Inland Waters Aquaculture Research Center (Ghazian Station). With special thanks to Moradi for facilities provided and assistance given.

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