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Histological studies of ovarian development of the Japanese Threadfin Bream, *Nemipterus japonicus*, in the Northern of Persian Gulf

*[1]M. Kerdgari; [2]T. Valinassab; [2]Sh. Jamili

[1] Islamic Azad University Bandar Abbas Branch, P.O.Box:7467147158 Bandar Abbas, Iran
[2] Iranian Fisheries Research Organization, PO Box: 14155-6116, Tehran, Iran

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ABSTRACT: In the present study, a total of 595 specimens collected monthly in the northern part of Persian Gulf between November 2006 and October 2007 were examined by routine macroscopic and histological techniques. Monthly changes in the gonadosomatic index showed the spawning season extended within 2 peaks, from April- May and September. The maturity stages are morphologically separated according to the changes in size, color and shape of the ovaries. Histologically, the oocytes development described the following stages; immature period (oogonia, early perinucleolus stage and late perinucleolus stage) maturation period (vacuolization stage, yolk granule stage, vitellogenic stage, germinal vesicle migration stage and mature yolk stage). The simultaneous presence of oocytes within ovaries indicated that this species is a batch spawner.

Keywords: *Nemipterus japonicas; maturity; histology; Persian Gulf*

INTRODUCTION

The Nemipteridae are marine perciformes that occur in the tropical-subtropical Indo-West Pacific. The genus *Nemipterus* is one of five genera belonging to the nemipteridae family and comprises 26 species (Russell, 1990). Nine species are found in Persian Gulf (Carpenter *et al.*, 1997). The Japanese threadfin bream, *Nemipterus japonicus* (Bloch, 1791), is small to moderate –sized fish found on mud or sandy bottoms in 5 to 80 m, usually in schools (Russell, 1990). This species constitutes an important part of the trawl catch in the South China Sea (Eggleston, 1972; Lee, 1974; Weber and Jothy, 1977), Andaman Sea (Senta and tan, 1975), W. Bay of Bengal (Krishnamoorthi, 1971), Persian Gulf and Oman Sea (Valinassab *et al.*, 2006). Some information is available on the population dynamics, food habits and feeding, reproductive biology, morphology and parasitological investigation of *N. japonicus* (Eggleston, 1972; Murty, 1984; Vivekanandan and James, 1986; Russell, 1990; Bakhsh, 1994; Zacharia, 1998; Rajkumar *et al.*, 2003; Manojkumar, 2004; Ghaem Maghami *et al.*, 2008 and Kerdgari *et al.*, 2009).

The purpose of the present study was to describe oocyte development using macroscopic and histological examination and determine ovarian development stages of *N. japonicus* in the northern of Persian Gulf.

We use only females because they are better indicators of the spawning season than males and also the oocytes determine the synthesis and maintenance of embryo storages (Santos *et al.*, 2005).

MATERIALS AND METHODS

Monthly samples were collected during the period from November 2006 and October 2007. A total of 360 females ranging from 110 – 263 mm in fork length (FL) and weighing 21.7 – 325.7 g. (BW) were caught by bottom trawlers in the northern part of Persian Gulf (Fig. 1). Fork length was measured to the nearest 1mm; body weight was weighed to the nearest 0.1g. Then, the ovaries were removed and weighed to the nearest 0.01g. The ovarian maturation was assessed by microscopic and macroscopic observation.

Small pieces of ovaries were fixed in 10% formalin buffer solution, dehydrated in alcohol, embedded in paraffin wax, sectioned at 6 µm thicknesses, and stained with hematoxylin and eosin (H&E) and examined by microscope. The description of each oocyte development stage was made according to terminology proposed by EL- Halfawy *et al.* (2007).

*Corresponding Author Email: *m_kerdgar@yahoo.com*

The Gonado-Somatic Index (GSI) was obtained according to the equation:

GSI % = gonad weight (g) × 100/ Body weight (g)

The gonadosomatic index or maturity index is an indirect method for estimating spawning season of a species (Biswas, 1993).

RESULTS AND DISCUSSION

Monthly variations in GSI were quite apparent (Fig. 2). Maximum values in April (2.70) and September (1.84). Significant differences were detected for GSI among months, according to ANOVA ($p < 0.05$). These cyclic changes in GSI indicate two peaks per year (April-May and September).

Morphological characteristics of ovaries

The maturity stages were morphologically separated according to the changes in size, colour and shape of the ovaries.

Ovarian development stages were assessed macroscopically based on the six-point scale (Table 1).

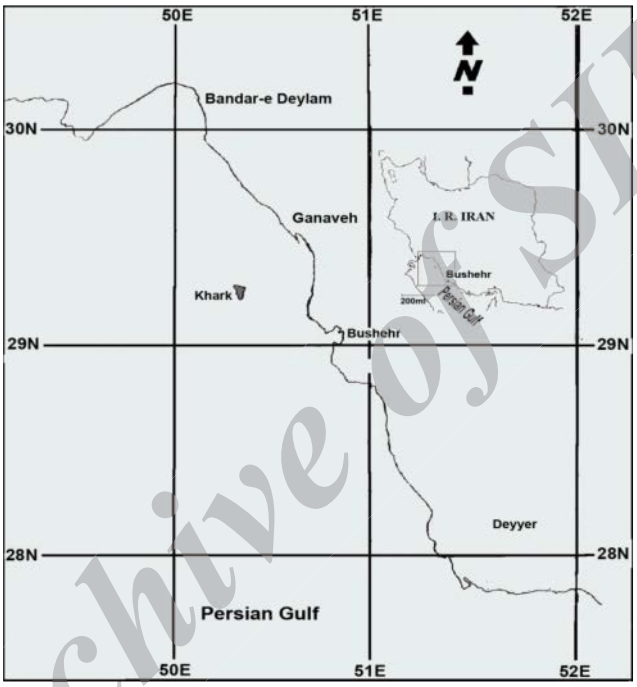

Fig. 1: Position of sampling site

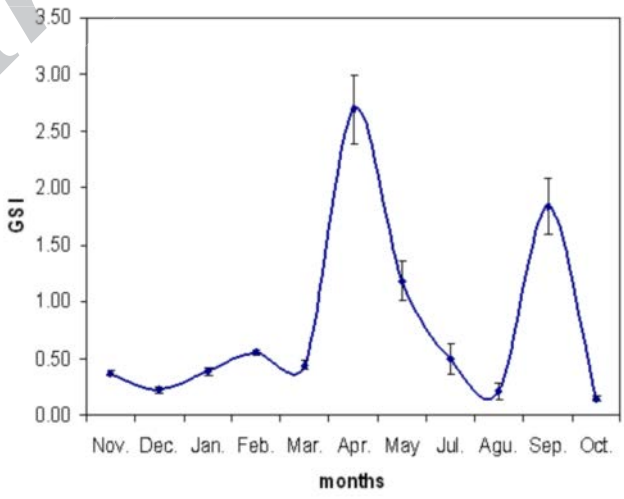

Fig. 2: Gonadosomatic Index variation of *N. japonicus* in the northern of Persian Gulf (2006-2007)

Table 1: macroscopic criteria for assessing of ovarian development stages in female *N. japonicus*

Reproductive stage	Macroscopic examination
I	Gonads are small, filiform and translucent.
II	Ovaries are larger (like band) and yellowish in colour, oocytes not visible to the naked eye (Figs.3, 7).
III	Ovaries are larger than previous stage, pinkish in colour, oocytes distinguishable (Figs.4, 8).
IV	Ovaries are enlarged and occupy 2/3 of body cavity. Pink in colour, well-developed red blood vessels. Oocytes are large and translucent (Figs.5, 9).
V	Ovaries are swell and elastic, occupying the whole body cavity, pink-yellow in colour, oocytes are hydrated and transparent and free in the ovarian lumen (Figs.6, 10).
VI	This stage is not described because not present in the samples.

Fig. 3: Stage II of ovary

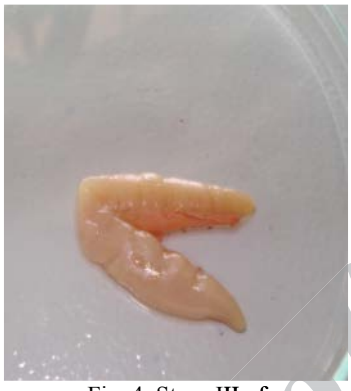

Fig. 4: Stage III of ovary

Fig. 5: Stage IV of ovary

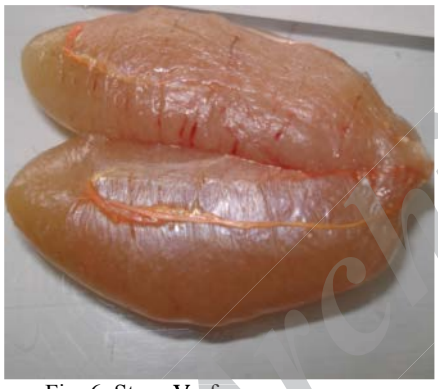

Fig. 6: Stage V of ovary

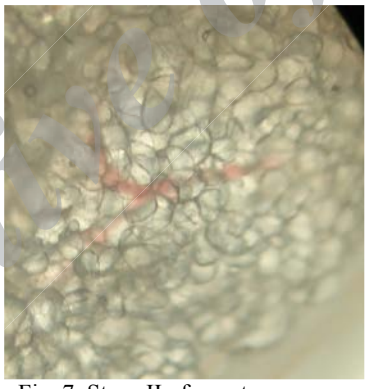

Fig. 7: Stage II of oocyte.

F ig. 8: Stage III of oocyte.

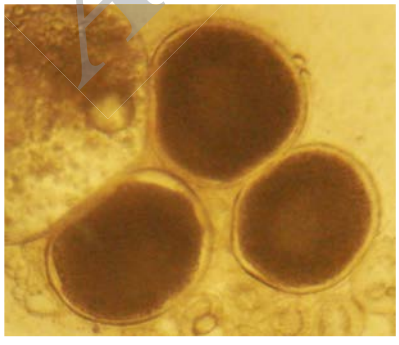

Fig. 9: Stage IV of oocyte.

Fig. 10: Stage V of oocyte.

Histological characteristics of ovaries

Eight oocyte development stages were identified for the *N. japonicus*, and described as follows:

1- Immature period (First growth phase)

Oogonia

Oogonia were very minute; spherical (about 4.36 µm in average) with relatively clear cytoplasm. They appeared singly or in small nests (Fig. 11).

Early perinucleolus stage
The oocytes were polygonal (about 60.50 µm in average), with strongly basophilic cytoplasm, large nucleus (about 33.12 µm in average) and a few number of nucleoli about 2 to 4 in the periphery of the nucleus (Fig. 12).

Late perinucleolus stage
The oocytes reached to 76.94 µm in average. The nucleoli located in the peripheral of the nucleus with a number of 5 to 12 (Fig. 13).

2- Maturation period (Second growth phase)
Vaculization stage
During this stage yolk vacuoles which appeared first in the periphery of the cytoplasm, increased in number and size dispersing throughout the cytoplasm. The zona radiata was observed between the oocyte and follicular epithelium. The oocytes reached to 135.84 µm in average (Fig. 14).

Yolk granule stage
This stage was characterized by the appearance of yolk granules in the periphery of the cytoplasm. As growth proceeds, the diameter of the oocyte increased to 162.42 µm in average and the nucleus diameter varied from 70 to 90 µm. The thickness of zona radiata became 4.54 µm and follicular epithelium measured 2.1 µm. The nucleoli reached 12 in number (Fig. 15).

Vitellogenic stage
In this stage, yolk accumulation proceeded rapidly and increased the oocyte diameter to 215 µm in average and the nucleus appeared with diameter 70 µm in average. Yolk vesicles occupied mostly the total volume of the cytoplasm of oocytes. The thickness of the zona radiata and follicular epithelium were measured 8.64 µm and 2.8 µm, respectively. The vacuoles diameter varied from 6 µm to 8 µm. (Fig. 16).

Germinal vesicle migration stage
During this stage the nucleus was migrating towards the periphery of the oocyte. The diameter of the oocyte measured between 220- 278 µm, the nucleus diameter measured 65 µm. The nucleoli reached from 11-16 in numbers and varied in diameter from 3-9 µm. The zona radiata and follicular epithelium were measured 11 µm and 4.87 µm, respectively. At the end of this stage the nucleus became amoeboid in shape (Fig. 17).

Mature yolk stage
At this stage, oocytes were the largest ovarian cell (285-315 µm). The zona radiata became more conspicuous (14 µm). The nucleus was not visible due to disintegration of the nuclear membrane and dispersion of its contents into the cytoplasm. The yolk vesicles fused, forming a yolk mass and the vacuoles fused, forming a large vacuole, which was the characteristic feature for the pelagic ova (Figs. 18, 19).
Atretic oocyte
Due to the activity of ovary, some oocytes fail to be ovulated reabsorbed and become atretic. This degeneration pattern was observed in second growth phase of oocyte development in *N. japonicus* (Fig. 20).

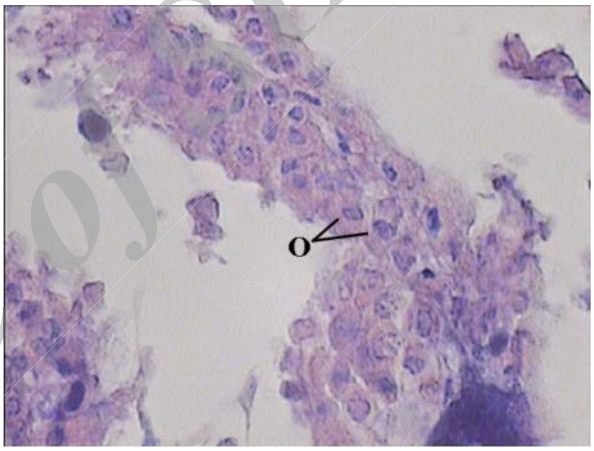

Fig. 11: Oogonia (O) (H&E x2000)

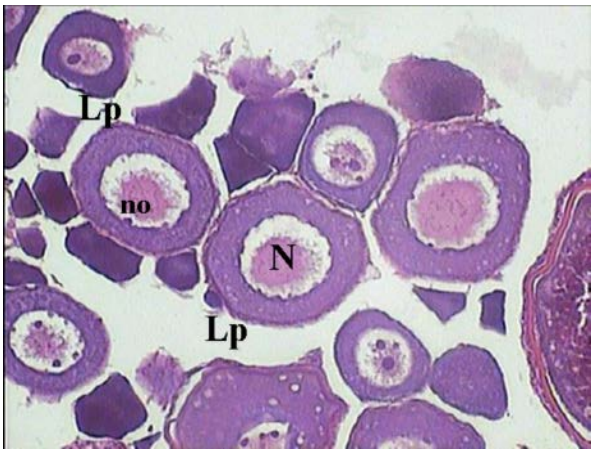

Fig. 13: Late perinucleolus stage (Lp), nucleus (N) and nucleolus (no) (H&E x500)

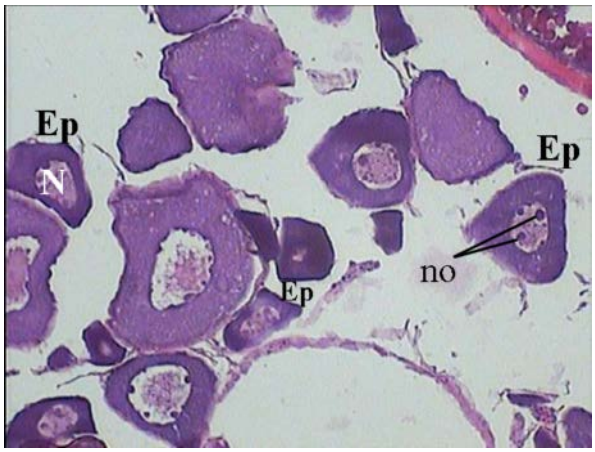

Fig. 12: Early perinucleolus stage (Ep), nucleus (N) and nucleolus (no) (H&E x500)

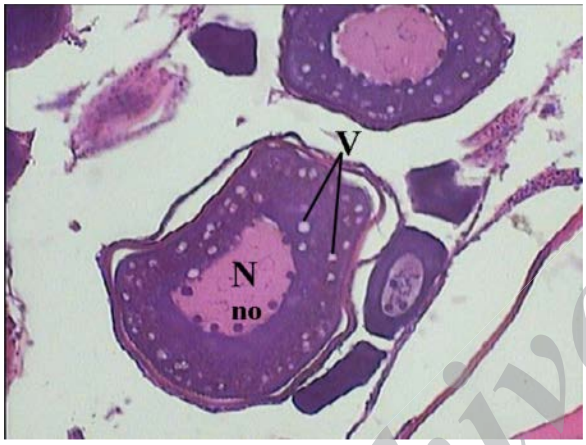

Fig. 14: Vaculization stage, nucleus (N), nucleolus (no) and vacuoles (V) (H&E x500)

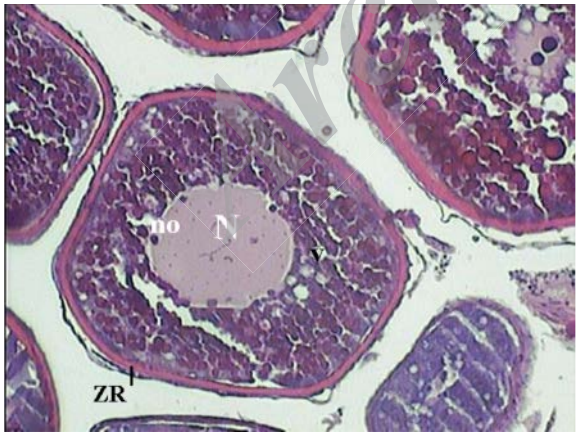

Fig. 15: Yolk granule stage, nucleus (N), nucleolus (no), vacuoles (V) and zona radiata (ZR) (H&E x500)

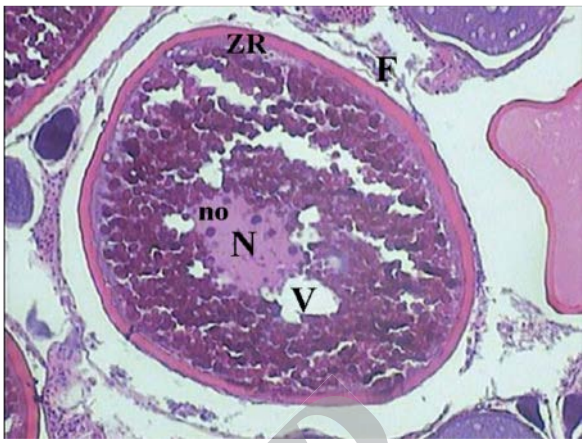

Fig. 17: Germinal vesicle migration stage, nucleus (N), nucleolus (no), vacuoles (V), zona radiate (ZR) and epithelial follicle (F) (H&E x500)

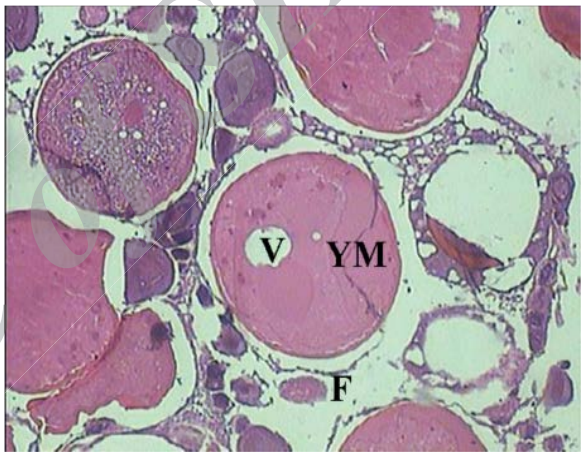

Fig. 19: Mature yolk stage, vacuoles (V), yolk masss (YM) and epithelial follicle (F) (H&E x200).

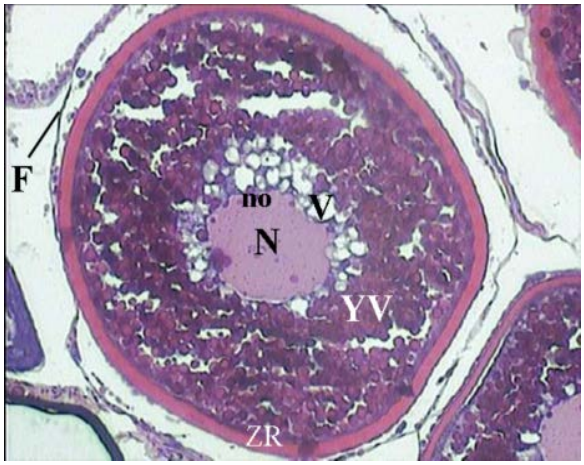

Fig. 16: Vitellogenic stage, nucleus (N), nucleolus (no), vacuoles (V), yolk vesicles (YV), zona radiate (ZR) and epithelial follicle (F) (H&E x500).

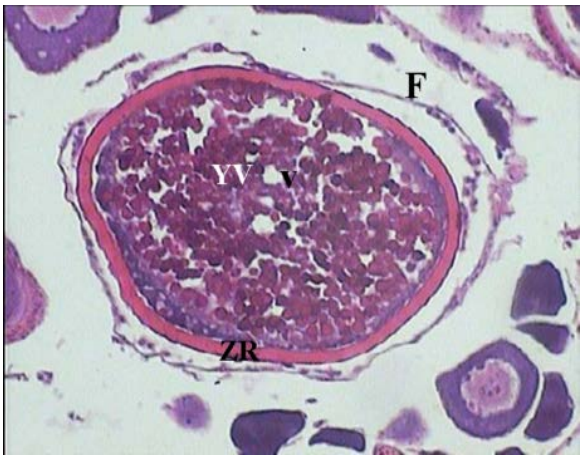

Fig. 18: Mature yolk stage, vacuoles (V), yolk vesicles (YV), zona radiate (ZR) and epithelial follicle (F) (H&E x500).

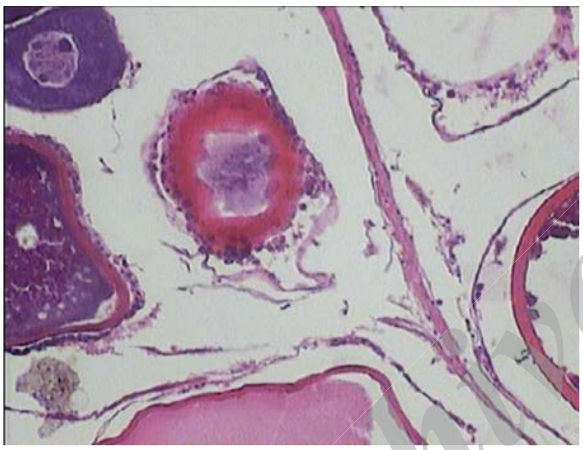
Fig. 20: Atretic oocyte (H&E x500).

CONCLUSION

The GSI has been widely used as an indicator of the spawning period in fishes (Santos *et al.*, 2005). The increase in GSI during the period of ovarian maturation is mainly due to the deposition of large amount of proteins and lipids in the developing eggs (Htun-Han, 1978). In the mature stage of *Nemipterus japonicus*, the GSI reaches the highest values in April (2.70) and September (1.84) due to accentuated volume of gonads, which occupy almost the whole of body cavity. Because of this sudden increase in ovarian weight in April-May and September, can be said that *N. japonicus* in the northern of Persian Gulf is a spring and autumnal spawner and main spawning occurs in spring season (Kerdgari *et al.*, 2009).

N. japonicus ovaries are classified as cystovarian (Hoar, 1969), as they present central and continuum lumen with the oviduct extending toward the urogenital aperture. In histological sections, oocytes of different development stages were presented within ovaries of *N. japonicus*. This type of ovary is known as asynchronous (Marza, 1938) and this species is a batch spawner. Also fishes that spawn in batches show the asynchrony type of ovarian development. This reproductive strategy decrease competition for sites and feeding resources for juveniles.

Oocyte growth follows a similar general pattern in most teleosts (Maddock and Burton, 1999; Kunckey and Sivakumaran, 2001; Shirali *et al.*, 2011). In present investigation there are two developmental phases of the oocyte growth, the primary growth phase and secondary growth phase. These phases were investigated by many authors (Latif and Saddy, 1973; Guraya *et al.*, 1975; Ramadan et al., 1978; EL-Garabawy and Abdel-Aziz, 1988; EL-Gharabawy, 1996; EL-Halfawy *et al.*, 2007).

Oogonia, the initial cells taking part in oogenesis, divide mitotically and form primary oocytes. Oocytes of *N. japonicus* in early perinucleolus stage are polygonal and nucleoli are varied in number from 2-4(Fig.12). In late perinucleolus stage, oocytes growing rapidly and the nucleoli increased and arranged in the periphery of the nucleus (Fig.13). Increasing the number of nucleoli associated with increasing RNA synthesis that is essential to the oocyte development because it is a complex cell differentiation process involving the formation of numerous nucleoli, development of nuclear and cytoplasmic inclusion and accumulation of cellular organelles (Narahara, 1991).

The secondary growth phase or maturation period is characterized by vacuoles apparently empty at the periphery of the cytoplasm, and containing endogen oil droplets. In this stage the zona radiata and follicular layer became visible with light microscope. As oocytes grow, the zona radiata and follicular layer increase in thickness and become more obvious. The role of the follicle cells in the oocytes is to form an active part in the transfer of proteins and other nutrients from the blood to the developing egg (EL-Gamal, 2003; EL-Halfawy *et al.*, 2007).The zona radiata controls the passage of substances to the interior of the oocytes during vitelogenesis, protects the oocytes against physical injury and promotes adherence of the egg to the substrate following ovulation (Agostinho *et al.*, 1987).

Vitellogenesis is one of the most important reproductive phenomenons in egg-laying animals. Vitellogenin is synthesized in the liver under hormonal influence and is deposited in growing oocytes as yolk protein, which serves as building and energy material after fertilization during embryogenesis till hatchling start feeding (Arockiaraj *et al.*, 2004). During exogen vitellogenesis, a remarkable oocyte development occurs and yolk

granules become larger and occupy the whole of cytoplasm (Santos *et al*., 2005).

At the ripening stage of oocyte, the migration of the nucleus to animal pole occurs (Zorica *et al*., 2005; EL-Halfawy *et al*., 2007).The nucleus was not visible due to disintegration of the nuclear membrane and dispersion of its contents into the cytoplasm. The yolk granules fused, forming a continuous mass of fluid yolk. This type of oocyte is called a hydrated oocyte (Hunter and Macewicz, 1985) and spawning begins with its formation (Hunter *et al*., 1986). In *N.japonicus* this stage was observed in April- May and September (Fig.19).

The degenerative phenomenon that occurs in the ovary (atresia) can happen in any phase of the reproductive cycle. However, in *N. japonicas,* this was observed in second growth phase while GSI was high. High frequencies of atresia may caused by environmental stress such as food shortages, pathologies, photoperiod and changes in temperature (Santos *et al*., 2005; EL-Halfawy et al., 2007). According to Palmer (1995), atresia in mature oocytes may decrease the reproductive potential of a species.

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