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Vitellogenesis in the Penaeid Prawn *Metapenaeus dobsoni* (Miers)

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Abstract

The process of vitellogenesis in the penaeid prawn *Metapenaeus dobsoni* (Miers) was studied histologically. The vitellogenesis could be broadly differentiated into 5 stages *viz.*, previtellogenic, early vitellogenic, late vitellogenic, vitellogenic and spent stages which correspond to the five maturity stages recognised in the species. The microscopic details of different stages of vitellognesis are described in this paper.

Introduction

The reproductive physiology of crustaceans has been extensively studied all over the world and more interest is evinced along this line in recent years on account of the importance in broodstock management of cultivable species. Comprehensive accounts on the oogenesis have been given by Raven (1961) and Adiyodi and Subramaniam (1983). According to Papathanasiou and King (1984), the process of vitellogenesis takes place in three distinct stages. First, a stage of germ cell division and formation of oogonia, then a stage of yolk deposition (vitellogenesis) and finally a post-vitellogenic stage. In the present study, vitellogenesis of the species grown in brackishwater environment is studied for the first time.

Materials and Methods

The gonads from prawns in different maturity stages were dissected out and the middle lobe of oavary fixed in Boins fluid in separate vails for 24-48 hrs. The samples were washed throughly in running water to remove excess picric acid. They were then dehydrated using ascending alcohol series (30-100% ethanol) and cleared in 2 changes of xylene. The tissues were then transferred through two changes of moulten was (Paraffin wax with Cresin, M.P. 58-60°C) and blocks prepared using paper boats. Serial sections of blocks were cut at approximately 5-8µm thickness, stained using Harrys Hematoxylin and counter stained with 1% aqueous Eosin solution and mounted in DPX mountant.

Results and Discussion

The process of oogenesis is associated with the oocyte levelopemnt and yolk accumulation in a graded manner. The ovary is a lobular organ. In prawns of size less than 60 mm, the varian lobe is occupied by primary and secondary oogonial ells with some of them getting transformed to formative stage f follicle cells (Fig.1a). In prawns above 65 mm size, the minal zone or germogen or germanium was restricted to a all region of the innermost ovarian layer (Fig. 1b). This zone roliferation was observed to be present in all maturity stages. Very small primary oogonial cells appear as thick mass in proliferative zone with distinct nucleus without clear cytoplasmic boundaries. These primary oogonial cells undergo mitotic divisions to form relatively large secondary oogonial cells which are more centrally placed. These cells possess a faintly visible cytoplasmic boundary with clear cytoplasm and nucleus. The follicle cells with less differentiation in their cellular structure surround secondary oogonial cells (fig. 1c). Based on changes manifested in cytoplasm and nucleus of the oocyte, the process of oogenesis was classified into five different stages, namely, pre-vitellogenic, early vitellogenic, late vitellogenic, vitellogenic and spent oocyte stages.

Pre-vitellogenic stage

This stage is characterised by the predominance of oogonia and primary oocytes (Fig. 1b). The secondary oogonial cells undergo meiotic division to form primary oocytes. The primary oocytes in the chromatin nucleolus stage are larger than the secondary oogonial cells with clear cytoplasmic boundaries and uniformly distributed chromatin network. Relatively larger perinucleolar oocyte having several nucleoli at the periphery of the nucleoplasm are also seen in this stage (Fig.1c). The nucleoli vary from 2-8 in number. Some oocytes with yolk nucleus in the peripheral region of cytoplasm are also noticed (Fig. 1d). The cytoplasm in all these primary oocytes is deeply basophilic, homogeneous and granular. The follicle cells differentiate into cuboid or hypertrophoied in shape and start surrounding the slightly larger oocytes.

Early vitellogenic stage

The oocytes increase in size rapidly and assume a size range of 0.19-0.32 mm. The oocytes are almost spherical in shape, with the cytoplasm moderately basophilic. The cytoplasm starts increasing in volume and becomes highly vaccuolated along the peripheral region (Fig. 1e). The granular appearance is due to the presence of vesicular yolk. The nucleus also gets enlarged and 15-20 nucleoli are seen along the peripheral region of the nucleoplasm. The chromatin net work is uniformly

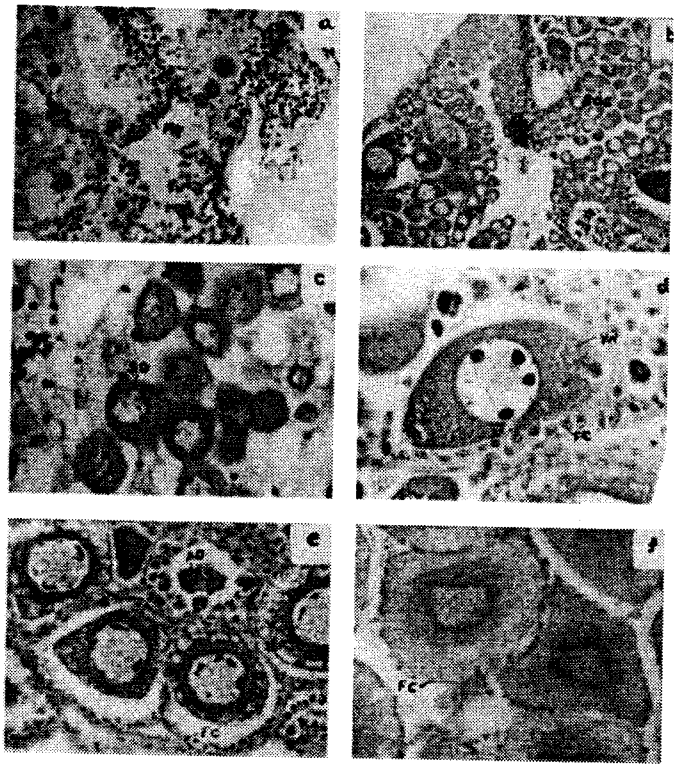

Fig. 1

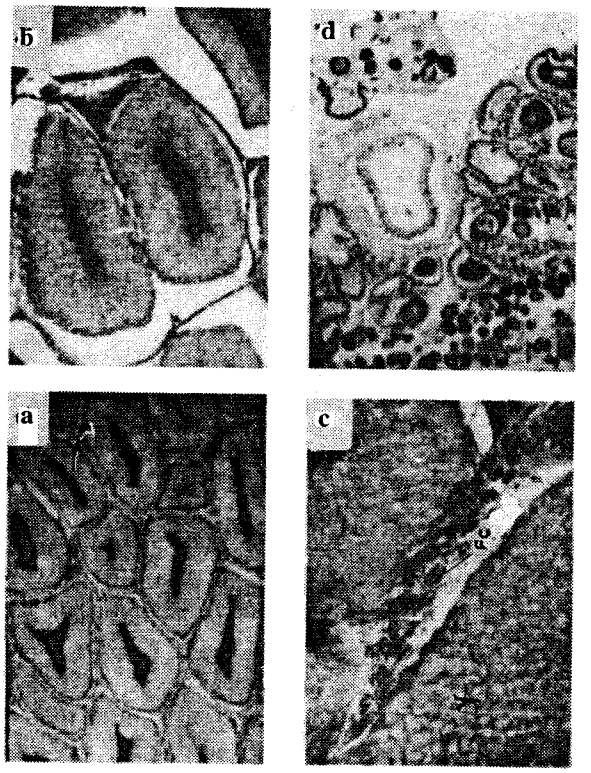

Fig. 2

PG - Primordial germ cells; SOC - Secondary oogonial cells; SO - Secondary oocytes; YN - Yolk nucleus; FC - Follicle cells; AO - Atretic oocyte; Y - Yolk granules.

distributed. The vaccuolated cuboid shaped follicle cells form a conspicuous band around the oocyte (Fig. 1e), leaving a log of empty space between the oocyte wall and the inner follicular layer. Atretic cells are abundant from this stage onwards and are characterised by a reduction of the ooplasm through resorption by follicle cells which turn from cuboidal to spherical in shape and basophilic to partly eosinopholic in nature.

Late vitellogenic stage

The oocytes further increase in size and grow to 0.24 to 0.43 mm in diameter. The nucleus slightly becomes elongated. In this stage, the ovarian wall becomes thin (fig. 1f) and the maturing oocytes are compactly arranged. The follicle cells become elongated and very closely surround the oocytes like a narrow band. A very clear shift is noticed from basophilic nature of cytoplasm to the eosinophlic nature with the cytoplasm becoming granular. The peripheral region of the cytoplasm continues to be slightly vaccuolated in smaller oocytes. The yolk granules are seen concentrated around the nucleus with sparse distribution in the peripheral region. The nucleus is lightly elongated and has a large number of nucleoli arranged along the peripheral region of nuceloplasm (Fig. 1f). Atretic cells are seen in this stage also with fully reabsorbed follicle cells.

Vitellogenic stage

The ovaries in this stage are completely filled with Vittellogenic oocytes and the oocytes of pre and early vitellogenic stages become insignificant. The oocytes assume the largest size measuring 0.24-0.65 mm. They are oval in shape and characterised by the eosinopholic cytoplasm completely flooded with yolk granules (Fig. 2a). The nucleus gets elongated like a narrow band with clearly visible nucleoli along its periphery. The striking feature of this stage is that the number of nucleoli get drastically reduced and the nuclear material in some cases gets dispersed in the cytoplasm (Fig. 2b). The follicle cells are elongated and form a thin ring around the oocytes and some times appear like a loose thin ribbon around the oocytes (Fig. 2b, c). Atretic oocytes are also seen.

Spent oocytes

Structurally the oocytes of this stage are almost same as previtellogenic and early vitellogenic stage (Fig. 2d). The larger oocytes of early vitellogenic stages are seen in various stages of resorption with some completely resorbed. The empty rings of thick layer of follicle cells caused by the ratraction of follicle cells are also seen in large numbers. Proliferation of fresh batch of oocytes showing the characteristics of previtellogenic oocytes, like nuclear halo, is also observed (Fig. 2c). A few deeply stained irregular primary oocytes are also noticed. Atretic oocytes are generally seen in this stage.

A wide variation in the placement of germinal zone in the ovary of crustaceans have been reported by Adiyodi and Subramaniam (1983). In the present study, it was found that the germinal epithelium on the inner ovarian wall, producing a continuous crop of oogonia, is confirned to a certain well defined area which has been referred to as zone of proliferation (Gutsell, 1936). Similar observations have also been made by King (1948) in *P.setiferous*, Subramaniam (1965) in *P. indicus*, and Shaikhmahmud and Tembe (1958) in *P. stylifera*. The gradual

movement of oogonia towards the centre of the lumen of ovary during their transformation into primary and secondary oocytes is clearly demonstrated in this species, similar to the observations made by King (1948) in *P. setiferus*. Shaikhmahmud and Tembe (1958) reports that in *P. stylifera* young or immature ova present in the cenre of lumen move towards the periphery as they grow and become mature. As in other crustaceans, the process of oogenesis in *M. dobsoni* is completed in two phases. First, is the proliferative phase in which the primary oogonial cells undergo mitotic division forming secondary oogonial cells which after meiotic division give rise to primary oocytes. The second pahse is the differentiative phase wherein the immature ova accumulate yolk and develop into mature oocytes. More or less similar observations have been reported in many other decapod crustaceans (Adiyodi and Subramaniam, 1983; Yamo, 1988). The process of oogenesis in *M. dobsoni* closely resembles that of *M.ensis* (Yano, 1985) and *P. stylifera* (Shaikhmahmud and Tembe, 1958). The mature ova of *M. dobsoni* shows significant structural variation from those of genus Penaeus. the stricking difference is the absence of cortical rods (Duronslet *et. al.*, 1975) which are indicators of imminent spawning (Anderson *et. al.*, 1984). The absence of cortical rods is also noticed in *M. ensis* (Yano, 1985) and *P. stylifera* (Shaikhmahmud and Tembe, 1985). Clark *et al.*, (1973) demonstrated that in *P. aztecus* the cortical bodies are responsible for the jelly layer which surrounds the eggs during early development. In *M. dobsoni*, the absence of such jelly like substance can be attributed to the absence of cortical bodies. The basophilic reaction of cytoplasm of ova of pre and early vitellogenic stages and the gradual shift to acidophlic nature in late vitellogenic and vitellogenic oocytes noticed this species similar to that noticed in *P. setiferus* (King, 1948), *P. monodon* (Tan Fermin and Pudadera, 1989) and *P. indicus* (Mohamed, 1989). Dring the process of oogenesis in *M. dobsoni*, the number of nucleoli gradually get reduced and finally in the vitellogenic stage, the entire nuclear material gets dispersed in the cytoplasm (Fig. 2a, b). This process is very clearly noticed in this species than in other prawns of the genus Penaeus reported by many workers.

Folliculogenesis in *M. dobsoni* is observed to begin in pre-vitellogenic stage and complete in early vitellogenic stage. In previtellogenic stage, the round follicle cells (Fig. 1b) surround a large number of secondary oogonial cells and in the later stage with increase in volume of oocyte, the follicle cells encircle the oocytes individually (Fig. 1e). The round follicle cells gradually elongate and form a thin ribbon like covering to the ovum in the late vitellogenic and vitellogenic stages (Fig. 2a, b). According to charniaux - Cotton (1975), follicle cells facilitate vitellogenic activity by aiding in uptake to yolk protein form external sources.

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References

Adiyodi, G.R. and Subramaniam, T., 1983. Arthropod - Crustacea, In : "Reproductive Biology of Invertebrates". Vol. I. Oogenesis Oosorption. K.G. Adiyodi and R.G. Adiyodi (Eds.), Wieley, Chichester England. pp. 442-495.

Andersen, S.L., Chang, E.S. and Clark, W.H. Jr., 1984. Timing of post-vitellogenic ovarian changes in the ridgeback prawn *Sioyonia ingentis* (Panaeidae) determined by ovarian biospy. *Aquaculture* 42 : 257-271.

Charniaux - Cotton, 1975. Control hormonale de la differentiation sexuella et. de activate genitale chez les crustaceous Malacostracous - *Publ. Stz. Zool. Napoli* 39 : 480 - 509.

Clark, W.K. Jr., Talbot, P., Neal, R.A., Mock, C.R... and Salser, B.R., 1973. In-vitro fertilization with non-motile spermatozoa of the brown shrimp *Penaeus aztecus. Mar. Biol.*, 22: 353-354.

Duronslet, M.J., Yudin, A.I., Wheeler, R.S. and Clark, W.H. Jr., 1975. Light and fine structural studies of natural and artificially induced growth of penaeid shrimp. *Proc. World Maricult. Soc.*, 6: 105-122.

Gutsell, James, S., 1936. A study of the ovaries of the common shrimp *Penaeus setiferus* (Linn) with reference to the life history (Unpublished).

King, J.E., 1948. A study of the reproductive organs of the common marine shrimp *Penaeus setiferus* (Linn). *Biol. Bull.*, 94 : 244-262.

Mohamed, K.S., 1989. Studies on the reproductive endocrinology of the penaeid prawn, *Penaeus indicus* (H. Milne Edesards). Ph.D. Thesis, Cochin University of Science and Technology, 255 pp.

Papathanassiov, E. and King, P.E., 1984. Ultrastructural studies on gametogenesis of the prawn *Palaemon serratus* (Pannant). 1-Oogenesis. *Acta. Zool. (Stock)*, 65(1): 17-31.

Raven, C.P., 1961. Oogenesis. The storage of developmental information. Pergamen Press, New York, 274 pp.

Shaikhmahmud, F.S. and Tembe, V.B., 1958. Study of Bombay prawns. The reproductive organs of Parapenaeopsis stylifera (M. Edwards). *J. Univ. Bombay*, 27(3) : 99-100.

Subramaniam, M., 1965. Lunar, diurnal and tidal periodicity in relation to the prawn abundance and migration in the Godavari estuarine systems. *Fishery Technol. Ernakulam*, 2(1): 26-33.

Tan Fermin and Pudadera, R.A., 1989. Ovarian maturation stages of the wild giant tiger prawn, Penaeus monodon Fabricius. *Aquaculture*. 77 : 229-242.

Yano, I., 1985. Induced ovarian maturation and spawning in Greasyback shrijp, *Metapenaeus ensis* by progesterone. *Aquaculture*, 47 : 223-229.

Yano, I., 1988. Oocyte development in the Kuruma prawn *Penaeus japonicus, Comp. Biochem. Physiol.*, 86 : 213-218.