

VITELLOGENESIS IN THE INDIAN WHITE PRAWN *PENAEUS INDICUS* (CRUSTACEA: DECAPODA: PENAEIDAE)

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ABSTRACT

The process of vitellogenesis in *Penaeus indicus* has been investigated using histological, histochemical, and electron microscopic methods. Based on the changes evident in the cytoplasm and nucleus, five vitellogenic phases are recognized. Histochemical studies revealed the yolk to be a glycolipoprotein complex, with proteins being the first to be associated with the yolk complex, followed by carbohydrates and finally lipids. Yolk formation apparently takes place by the selective deposition and sequestration of organic material from within (autosynthesis) and without (heterosynthesis). During the early vitellogenic phase the emphasis is on intraoocytic synthesis of proteins aided by the extrusion of RNA-rich nucleolar material into the cytoplasm through nuclear pores and the presence of abundant free ribosomes, rough endoplasmic reticulum, and mitochondria. From the late vitellogenic phase onwards extraoocytic sequestration of organic reserves, especially lipids, is prevalent through the formation of micropinocytotic vesicles on the oolemma. The latter process appears to be predominant.

Knowledge about cytological changes which occur during gametogenesis in Arthropoda in general and in insects in particular is extensive. However, such studies are very few in Crustacea and among penaeids they are scanty. Most investigations on penaeid prawns have been restricted to the description of spawning seasons of several commercially important species (Rao, 1968; Thomas, 1974; Penn, 1980; Kennedy and Barber, 1981). Using histological techniques, the anatomy of the reproductive organs and maturity stages has been described in prawns (Hudinaga, 1942; King, 1948; Shaikhmahmud and Tembe, 1958; Subrahmanyam, 1965; Duronslet *et al.*, 1975; Yano, 1988; Tan-Fermin and Pudadera, 1989; Qunitio and Millamena, 1992). However, the process of vitellogenesis in prawns has not been subjected to detailed investigations.

The present investigation, therefore, aimed to elicit the process and events leading to maturation of the ovary in the Indian white prawn *Penaeus indicus*. The process of vitellogenesis has been described using light and electron microscopic

techniques. A histochemical study of the ovary has also been carried out to determine the sequence in which yolk substances are synthesized or sequestered in the oocytes and also the nature of the yolk material.

MATERIALS AND METHODS

Penaeus indicus in the size range of 120–170 mm total length were collected from the sea off Kochi (SW coast of India) using short duration otter trawls. They were then carefully transported to the laboratory in transportation bags and kept in plastic pools with continuous aeration until use.

Female prawns in different maturity stages (I to V) identified on the basis of morphological (Rao, 1968) and histological (Subrahmanyam, 1965) characters were taken and their cephalothorax and abdomen were cut open to expose the ovary. The middle lobes of the ovary were cut and fixed in Bouin's fluid for 24–48 hrs. The tissues were then processed and embedded in paraffin and sections cut at 8 μ m were stained with Harris hematoxylin and eosin.

Histochemical tests

Tests with suitable controls were performed as per methods described in Pearse (1968). For detecting proteins (basic and acidic), amino acids (amino, sulphhydryl, disulphide, tyrosyl and tryptophanyl end groups and histones), nucleic acids (DNA and RNA), and carbohydrates (1,2 glycol groups, glycogen, glycoprotein, glycolipid, sulphated and non-sulphated acid mucopolysaccharides and neutral mucopolysaccharides), ovaries in different maturity stages were fixed in 10% neutral buffered formalin for 24–48 hrs. For lipid (neutral and acidic lipids, phospholipids, and unsaturated lipids) histochemistry, sections (10 μ m thick) of fresh ovaries were cut in an AO Histostat at -20°C .

Ultrastructural studies

Fresh ovaries in all maturity stages were fixed in cold 4% gluteraldehyde and post-fixed in 1% osmium tetroxide. They were then processed and embedded in Araldite. Ultrathin sections were cut and post-stained in uranyl acetate and lead citrate and examined in a JEOL-JEM 100CX II electron microscope at 80 KV.

RESULTS

Histological preparations of the ovary in different stages of maturity revealed the graded manner in which oocytes developed and accumulated yolk. Based on the changes evident in the cytoplasm and nucleus of the oocytes, the complete development of the oocytes was classified into five phases, namely, pre-vitellogenic, early vitellogenic, late vitellogenic, mature and spent oocytes (Tables 1, 2). These oocyte phases correspond to the maturity stages I to V described earlier (Rao, 1968; Subrahmanyam, 1965).

Table 2. Classification of maturity stages in female *P. indicus* based on colour of the ovary, GSI and oocyte diameter

Ovary stage	Colour and appearance of ovary	GSI \pm SD	Oocyte diameter $\mu \pm$ SD	Nucleus diameter $\mu \pm$ SD
STAGE I				
Immature ovary	Translucent smooth	0.435 \pm 0.272		
Primary oogonial cells			7.2 \pm 2.2	5.5 \pm 0.8
Secondary oogonial cells			21.4 \pm 4.3	13.2 \pm 1.8
Primary oocytes			39.1 \pm 10.0	20.9 \pm 6.3
STAGE II				
Early maturing ovary	Pale cream smooth-granular	2.207 \pm 0.619	125.8 \pm 22.6	44.2 \pm 7.6
STAGE III				
Late maturing ovary	Light green granular	4.527 \pm 1.499	187.3 \pm 19.4	60.0 \pm 7.0
STAGE IV				
Mature/ripe ovary	Dark green granular	7.312 \pm 1.059	241.3 \pm 16.7	66.4 \pm 3.6
STAGE V				
Spent/spent-recovering ovary	Pale cream granular-flaccid	1.042 \pm 0.413	25.1 \pm 4.9	17.6 \pm 2.6

The ovary was encompassed by a thin ovarian wall composed of epithelial cells (Fig. 1). The germinal zone was observed in all sections as a thin band along the innermost layer of the ventral ovarian wall (Fig. 2) and it persisted in all maturity stages. The smaller primary oogonial cells were found at the periphery of the germinal zone and the larger secondary oogonial cells were distributed towards the centre of the ovary in a graded manner (Fig. 2). Oogonial cells had pale eosinophilic cytoplasm with a large, conspicuous nucleus having 10 to 20 nucleoli (Fig. 3). Oogonial cells were devoid of follicle cell covering.

Pre-vitellogenic oocytes (PVO)

The PVO had a strikingly basophilic cytoplasm with increased cytoplasmic volume (Fig. 1). In a stage I ovary these oocytes occupied the entire lumen in clusters surrounded by follicle cells. The nuclei were lightly stained with hematoxylin and had 5–10 darkly stained nucleoli along the peripheral margin. The follicle cells were highly vacuolated and tall with conspicuous nuclei (Fig. 1).

Ultrastructurally, the nucleoli of the PVO appeared as electron-dense bodies along the inner periphery of the nuclear wall (Fig. 4). Numerous gaps or nuclear pores were evident on the nuclear wall through which electron-dense nucleolar material was observed to diffuse into the cytoplasm. Organelles like mitochondria and endoplasmic reticulum (ER) were also evident.

Histochemical studies on the immature ovary showed that the nucleus and nucleoli of oogonial cells were devoid of any carbohydrate substances (Table 3). The thin rim of cytoplasm in the oogonial cells was positive to tests for glycogen

Table 1. Distinctive changes in the cytological structure of the oocytes in relation to vitellogenesis in *P. indicus*

Oocyte phase	Cytoplasm	Nucleus	Nucleoli	Follicle cells
Stage I Previtellogenic	Homogeneous, basophilic due to the presence of RNA; mitochondria and rough ER present.	Vesicular with numerous nuclear pores.	5-10; electron dense; arranged along peripheral margin of the nucleus.	Tall with conspicuous nuclei.
Stage II Early vitellogenic	Granular; perinuclear halo of nucleolar RNA; mitochondria and rough ER present; vesicular primary yolk.	Vesicular with nuclear pores.	5-10; arranged in a circular ring along the nuclear wall.	Decrease in cell height; hypertrophied nucleus and nucleoli.
Stage III Late vitellogenic	Granular and acidophilic; lipid globules and yolk platelets present. ER and mitochondria not apparent; oolemma with micropinocytotic vesicles.	Faintly stained.	Nucleoli number reduced to 2-3.	Flattened around oocyte; rough ER and mitochondria present.
Stage IV Mature	Full of yolk platelets; disulphide-rich cortical bodies with feathery matrix.	Faintly stained.	Not apparent.	Very flattened and stretched around oocyte.
Stage V Early vitellogenic (spent)	Deeply basophilic with signs of pycnosis.	Vesicular.	5-10; arranged along the perinuclear margin.	Intensely PAS positive and hypertrophied.

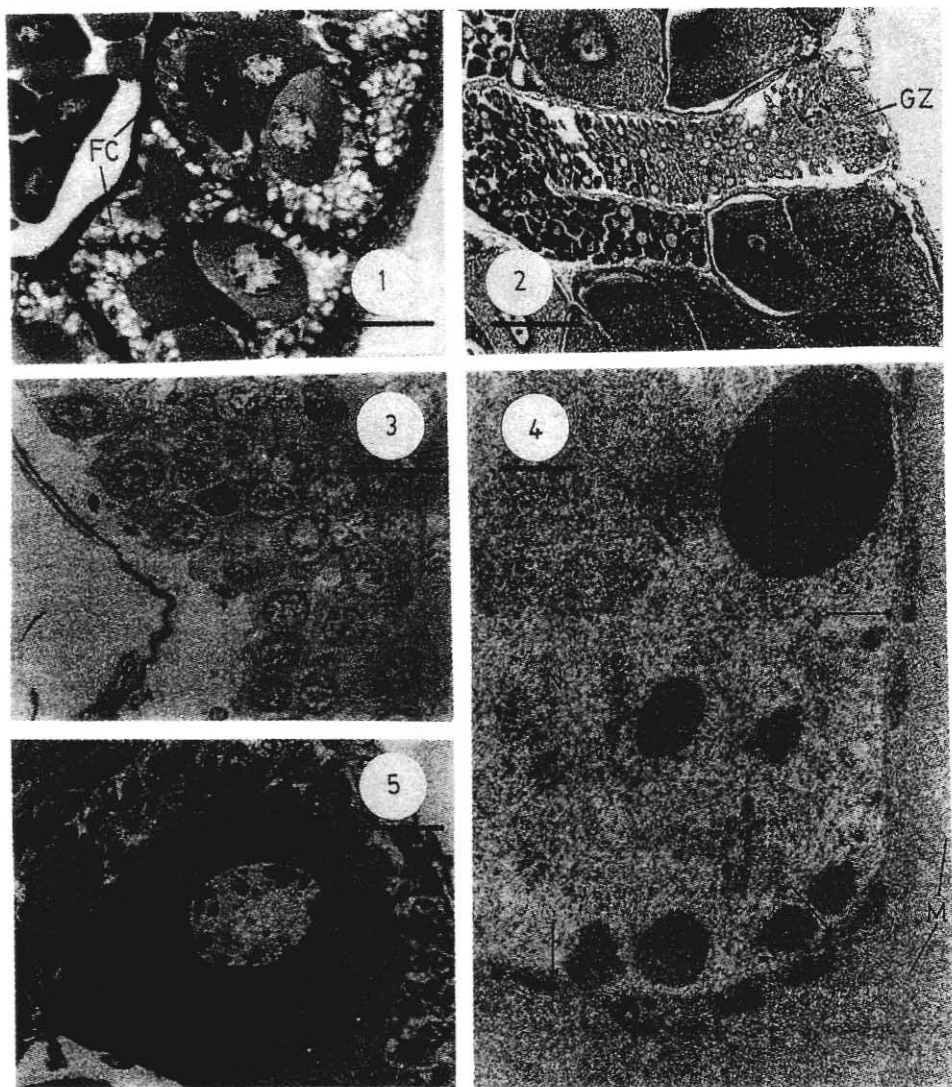


Fig. 1. Semi-thin section of a stage I ovary showing pre-vitellogenic oocytes surrounded by tall follicle cells (FC). Toluidine blue stain. Bar equals 50 μ m.

Fig. 2. Cross-section of the ovary showing ventro-medial position of the germinal zone (GZ). Toluidine blue stain. Bar equals 25 μ m.

Fig. 3. Semi-thin section of the germinal zone showing primary and secondary oogonial cells. Note the absence of follicle cells. Toluidine blue stain. Bar equals 25 μ m.

Fig. 4. Electron micrograph of a PVO showing electron-dense nucleoli (NUL) adjacent to the nuclear membrane. Arrows indicate pores through which the nucleolar material diffuses out. N—nucleus; M—mitochondria. Bar equals 1.0 μ m.

Fig. 5. Semi-thin section of early vitellogenic oocytes with perinuclear halo of nucleolar material and granular cytoplasm. The follicle cells (FC) are flattened and have hypertrophied nucleus. Toluidine blue stain. Bar equals 25 μ m.

Table 3. Histochemical responses of the ovary for carbohydrates during vitellogenic phases in *P. indicus*

Histochemical tests	Oogonial cells			PVO				EVO				LVO				MO				SO			
	CY	N	NUL	CY	N	NUL	FC	CY	N	NUL	FC	CY	N	NUL	FC	CY	N	FC	CB	CY	N	NUL	FC
Schiff alone	-	-	-	±	-	-	-	±	-	-	-	±	-	-	-	±	-	-	-	-	-	-	-
Periodic Acid																							
Schiff (PAS)	±	-	-	+	-	-	++	+++	-	-	+	+++	-	-	±	++	-	-	+	±	-	-	+++
Deamination	±	-	-	±	-	-	++	++	-	-	+	+	-	-	±	++	-	-	±	-	-	-	++
Acetylation	-	-	-	±	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
Deacetylation	±	-	-	+	-	-	++	+++	-	-	+	+++	-	-	±	++	-	-	+	±	-	-	+++
Delipidation	±	-	-	+	-	-	++	+++	-	-	±	++	-	-	±	+	-	-	+	±	-	-	+++
Diastase digestion	-	-	-	±	-	-	+	++	-	-	+	++	-	-	+	+	-	-	+	±	-	-	++
Best's Carmine test	+	-	-	+	-	+	++	++	+	++	+++	++	-	+	++	++	-	+	-	+	-	++	++
Diastase digestion	-	-	-	+	-	-	±	+	-	+	++	++	-	+	+	+	-	-	-	+	-	+	+
Alcian Blue																							
-C-E-C-Method																							
0.1 M	+	-	-	-	-	-	+	+	+	+	++	++	+	+	++	+	-	-	-	-	-	-	+++
0.2 M	-	-	-	+	-	-	-	+	++	-	++	+	±	-	+	+	-	-	+	-	-	-	++
0.6 M	-	-	-	-	-	-	-	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-
0.8 M	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1.0 M	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Bracco-Curti's test	+	-	-	++	-	-	+	++	+++	++	+	+	+	-	-	+	-	-	+	±	+	+	-

CY = cytoplasm; N = nucleus; NUL = nucleolus; CB = cortical bodies; FC = follicle cell; - = negative reaction; ± = very mild reaction of doubtful nature; + = positive reaction; ++ = strongly positive reaction; +++ = intense positive reaction.

and sulphated mucosubstances. The PVO had a similar chemical constitution apart from the increased positivity to periodic acid Schiff (PAS) test. The follicle cells were strongly positive to the PAS test due to the presence of 1,2 glycol groups.

The oogonial cells displayed intense positive reaction for proteins of both basic and acidic nature (Table 4). The ooplasm of PVO stained positively for amino, tyrosine, tryptophan, and disulphide groups. Both the ooplasm and the nucleoli were intensely pyroninophilic due to the presence of large amounts of RNA. Follicle cells stained positive to all classes of proteins excepting tyrosine and tryptophan. Oogonial cells as well as follicle cells were poor in lipids (Table 5), while PVO showed positive reaction to tests for acidic lipids and phospholipids.

Early vitellogenic oocytes (EVO)

The ooplasm of EVO was less basophilic and appeared granular (Fig. 5) due to the presence of vesicular primary yolk. The perinuclear halo of nucleolar material was present in this phase also, indicating diffusion of RNA-rich nucleolar material into the ooplasm. The follicular investment around the individual oocytes or folliculogenesis was completed during this phase. The follicle cells were considerably stretched and an apparent decrease in their height was noticed (Fig. 5). Follicle cells had conspicuous and hypertrophied nuclei and nucleoli.

Electron microscopic observations of the EVO revealed the abundance of mitochondria, rough ER, and free ribosomes in the ooplasm (Fig. 7). The primary yolk bodies were present in the form of swollen reticular elements. A nucleolonema was observed in the nucleoplasm (Fig. 6). This is a modified nucleolus with a filamentous appearance.

The ooplasm was intensely positive to the PAS test (Table 3), and this was only partly removed by deamination, while being totally removed by acetylation indicating the presence of 1,2 glycol groups and glycoproteins. Alcianophilia at low molar concentrations showed the presence of carboxylated and sulphated acid mucopolysaccharides (AMP) in the ooplasm. Follicle cells showed less PAS positivity and contained both carboxylated and sulphated AMPs.

Almost all classes of proteins tested were abundant in EVO ooplasm (Table 4). A decrease in positivity to pyronin was observed and the reaction was confined to the perinuclear region. The nucleoli, however, remained intensely pyroninophilic. The EVO ooplasm displayed an increase in positive reaction to tests for acidic and phospholipids (Table 5). Neutral lipids and lipids with unsaturated bonds were present in small amounts only.

Late vitellogenic oocytes (LVO)

Characteristic of this phase was the very granular cytoplasm which was now wholly eosinophilic. The nucleus was palely stained with hematoxylin and the nucleoli number greatly reduced. The granular nature of the cytoplasm was mainly due to the formation of dense yolk platelets and accumulation of lipid globules (Fig. 8). Fully formed yolk platelets were darkly stained, partly formed ones lightly

Table 4. Histochemical responses of the ovary for proteins and

Histochemical tests	Oogonial cells			PVO				EVO			
	CY	N	NUL	CY	N	NUL	FC	CY	N	NUL	FC
Mercuric Bromophenol Blue	+++	+	++	++	+	++	+	+++	++	+++	+++
Aq. Bromophenol Blue	+++	+	+	+	+	++	+	+++	+	++	+
Deamination	+	-	-	-	-	-	-	++	-	+	-
Aq. Toluidine Blue	++	-	-	++	-	+++	+	+	-	+++	+++
Methylation	-	-	-	-	-	-	-	-	-	-	-
Ferric Ferricyanide	+	-	-	+	++	++	+	+	++	+	++
Mercaptide	-	-	-	+	-	+	-	-	+	+	+
Performic Acid Alcian Blue	-	-	-	+	-	-	++	+	-	-	++
Alcian blue alone	-	-	-	-	-	-	+	-	-	-	±
Millon's test	++	+	+	+	-	±	-	++	-	+	+
Iodination	-	-	-	-	-	-	-	-	-	-	-
DMAB - Nitrite test	±	±	-	++(PN)	-	+	-	++	+	++	+
Formaldehyde	-	-	-	-	-	-	-	-	-	-	-
Ninhydrin - Schiff	++	-	+	+	-	+	+	++	-	++	++
Deamination	-	-	-	-	-	-	-	-	-	-	-
Methyl Green Pyronin	+(R)	±(G)	±(R)	+++ (R)	±(G)	+++ (R)	++ (R)	++ (R)	±(G)	+++ (R)	++ (G)
10% Perchloric acid	-	±(G)	-	-	±(G)	-	-	-	±(G)	±(R)	+(G)
Fuelgen Reaction	-	+++	±	-	++	+	+	-	±	±	++
10% Perchloric acid	-	-	-	-	-	-	-	-	-	-	-

PN = perinuclear, R = red, G = green, PP = peripheral.

stained, and lipid droplets unstained. The follicle cells appeared as a narrow band of flattened cells encompassing the oocytes.

Yolk platelets were spherical to oval in shape and electron dense (Fig. 9). The formation of the yolk platelets was apparently achieved through two sub-stages. The initial yolk vesicle was almost electron lucent and filled with tiny electron-dense flocculent granules. Through progressive differentiation these granules aggregated to form a moderately electron-dense yolk sphere which finally devolved into the yolk platelet (Fig. 9). The oolemma, which was smooth in the EVO phase, now appeared broken with the presence of numerous micropinocytotic vesicles (Fig. 10) in close apposition to the follicle cells. The follicle cells possessed a hypertrophied nucleus, rough ER, and mitochondria.

Yolk spheres and platelets were deeply PAS positive, denoting the presence of carbohydrate substances conjugated with proteins and lipids (Table 3). Yolk platelets were positive to all classes of proteins tested (Table 4). Positive reactions were observed in the peripheral and perinuclear regions of the ooplasm for -S-S and -SH group amino acids. Yolk spheres were negative to pyronin test for RNA. Follicle cells reacted strongly to tests for sulphur-containing amino acids. Yolk platelets were intensely sudanophilic and acid hematein positive indicating the abundance of lipids in general and phospholipids in particular (Table 5). Yolk bodies also showed the presence of acidic, neutral, and unsaturated lipids.

nucleic acids during vitellogenic phases in *P. indicus*

LVO				MO				SO			
CY	N	NUL	FC	CY	N	FC	CB	CY	N	NUL	FC
+++	+	+++	+	+++ (PN)	+	++	+(PP)	++	+	+++	+
+++	+	++	+	+++	+	+	±	+	-	+	-
++	-	±	-	++	-	-	-	-	-	-	-
+(PN)	-	+	+	-	+	++	-	+++	-	++	±
-	-	-	-	-	-	-	-	-	-	-	-
++ (PP)	+	-	++	++ (PP)	+	++	+++	+	-	+	++
+	-	-	-	++	-	-	+	-	-	±	+
++ (PP)	-	-	+++	+	±	++	+++	±	-	-	+++
-	-	-	+	-	-	+	-	-	-	-	+
++	-	±	++	++	-	+	+	±	-	+	-
-	-	-	-	-	-	-	-	-	-	-	-
+++	+	++	++	++	+	+	+	±	-	-	-
-	-	-	-	-	-	-	-	-	-	-	-
+++	+	++	+	++	+	-	+	±	-	+	-
±	-	-	-	-	-	-	-	-	-	-	-
± (R)	± (G)	+(R)	++ (G)	-	± (G)	+(G)	± (R)	-	± (G)	+(G)	++ (G)
-	±	-	+(G)	-	±	-	-	-	-	-	± (G)
-	±	-	++	-	-	+	-	-	+	+	++
-	-	-	-	-	-	-	-	-	-	-	-

Mature oocytes (MO)

Stage IV ovaries were filled with mature oocytes which were characterized by the abundance of yolk platelets filling the entire ooplasm and the appearance of elongated rod-like cortical bodies on its peripheral margin adjacent to the oolemma (Fig. 11). The nucleus was stained faintly and the nucleoli were not apparent at this stage. The follicle cells were further flattened and were present only as a thin covering.

The ultrastructure of LVO and MO were similar with the exception of the presence of cortical bodies in the latter. The cortical bodies exhibited a distinct substructure (Fig. 12). The matrices of these bodies were packed with minute, feather-like strands arranged randomly.

The yolk platelets in mature oocytes had a slightly reduced positive reaction to PAS and moreover the reaction was not wholly blocked by acetylation and diastase digestion (Table 3) due to the conjugated nature of the yolk (glycolipoprotein). The cortical bodies were weakly PAS positive and also positive to the test for sulphated AMP. The MO did not exhibit any major difference in the reactions to tests for proteins with that of LVO (Table 4). Interestingly, the cortical bodies were intensely positive to tests for disulphide and sulphhydryl groups. Reactions to lipid tests were also unchanged when compared to LVO (Table 5).

Spent oocytes (SO)

Oocytes of spawned prawns showed characters identical to those of PVO and EVO. The spent ovary displayed empty follicles, sites of oocyte resorption, and

Table 5. Histochemical responses of the ovary for lipids during vitellogenic phases in *P. indicus*

Histochemical tests	Oogonial cells			PVO				EVO				LVO				MO				SO			
	CY	N	NUL	CY	N	NUL	FC	CY	N	NUL	FC	CY	N	NUL	FC	CY	N	FC	CB	CY	N	NUL	FC
Sudan Black B	±	±	±	+(GB)	-	-	±	++	-	-	±	+++ (GB)	±	-	±	+++ (GB)	-	-	-	+	-	-	±
Delipidation	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Nile blue method		-	+	+++ (BL)	±	-	+(BL)	++ (BL)	-	±	+	++ (BL)	-	-	+	++ (BL)	-	-	-	+(BL)	±	±	+
Delipidation	±	-	-	+	±	-	-	-	-	-	±	±	-	-	-	+	-	±	±	-	-	-	±
Baker's Acid Hematein	+(BR)	±	+	++ (BB)	+	+	±	++ (BB)	-	-	+	+++ (BB)	-	-	+	+++ (BB)	-	+	+	+	+	+	±
Pyridine extraction	±	-	-	-	-	-	-	±	-	-	-	±	-	-	-	±	-	-	±	±	-	-	-
Oil Red O method	-	-	-	±	-	-	-	+	-	-	±	+++	-	-	-	+++	-	±	-	-	-	-	±
Delipidation	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
UV Schiff reaction	+	-	+	±	-	-	±	+	-	-	+	++	-	-	-	++	-	±	-	±	-	-	±
non UV	-	-	+	+	-	-	±	+	-	-	±	±	-	-	-	+	-	-	-	-	-	-	-

BL = blue, Br = brown, BB = blue black, GB = greyish black.

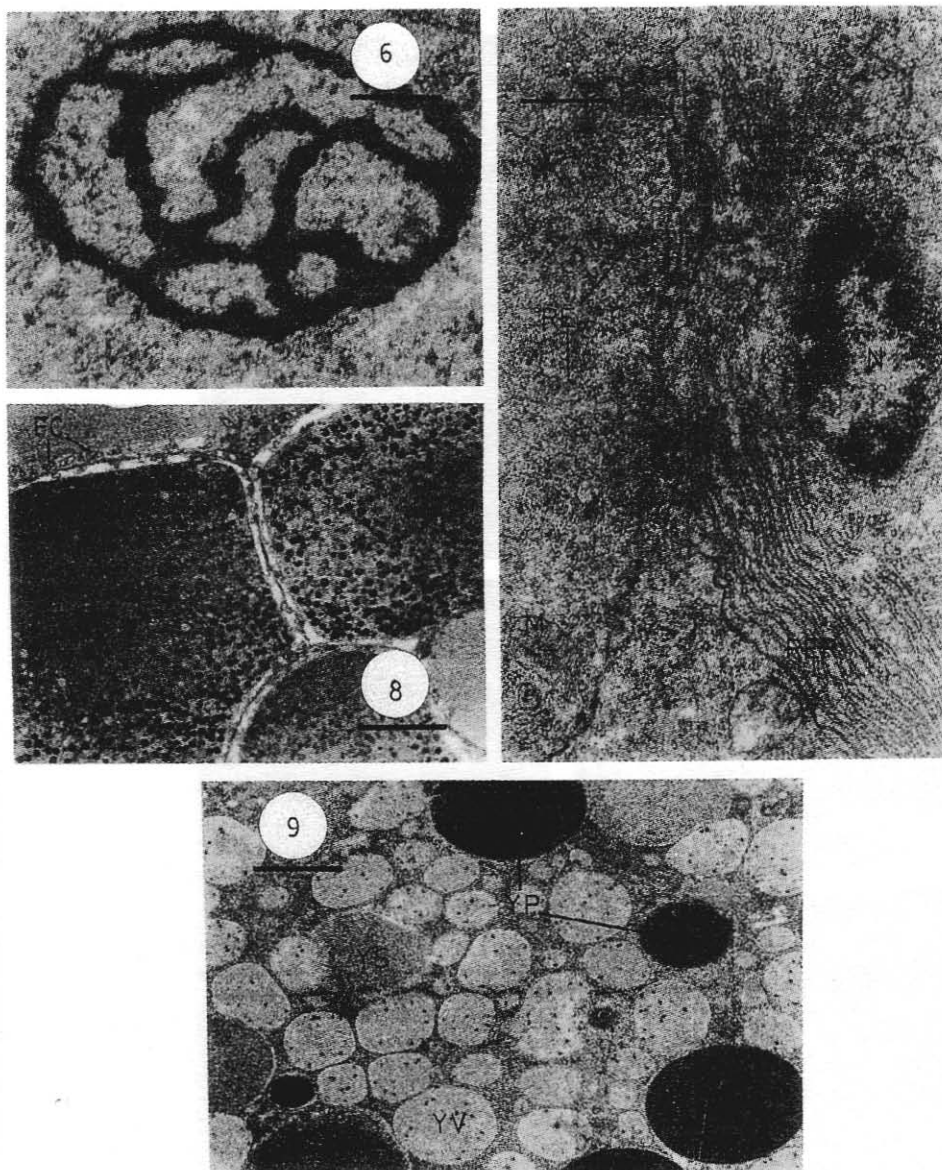


Fig. 6. A filamentous nucleolonema in the nucleoplasm of an early vitellogenic oocyte. Bar equals 0.5 μ m.

Fig. 7. Electron micrograph of the perinuclear region of an EVO displaying mitochondria (M), rough endoplasmic reticulum (RER), swollen reticular elements (RE), and numerous free ribosomes. N—nucleus. Bar equals 0.5 μ m.

Fig. 8. Semi-thin section of LVO showing darkly stained yolk platelets, lightly stained yolk vesicles, unstained lipid globules, and flattened follicle cells (FC). Toluidine blue stain. Bar equals 25 μ m.

Fig. 9. Electron micrograph of the cytoplasm of LVO showing the formation of yolk. The yolk vesicles (YV), yolk spheres (YS), and fully formed yolk platelets (YP) are evident. Bar equals 1.0 μ m.

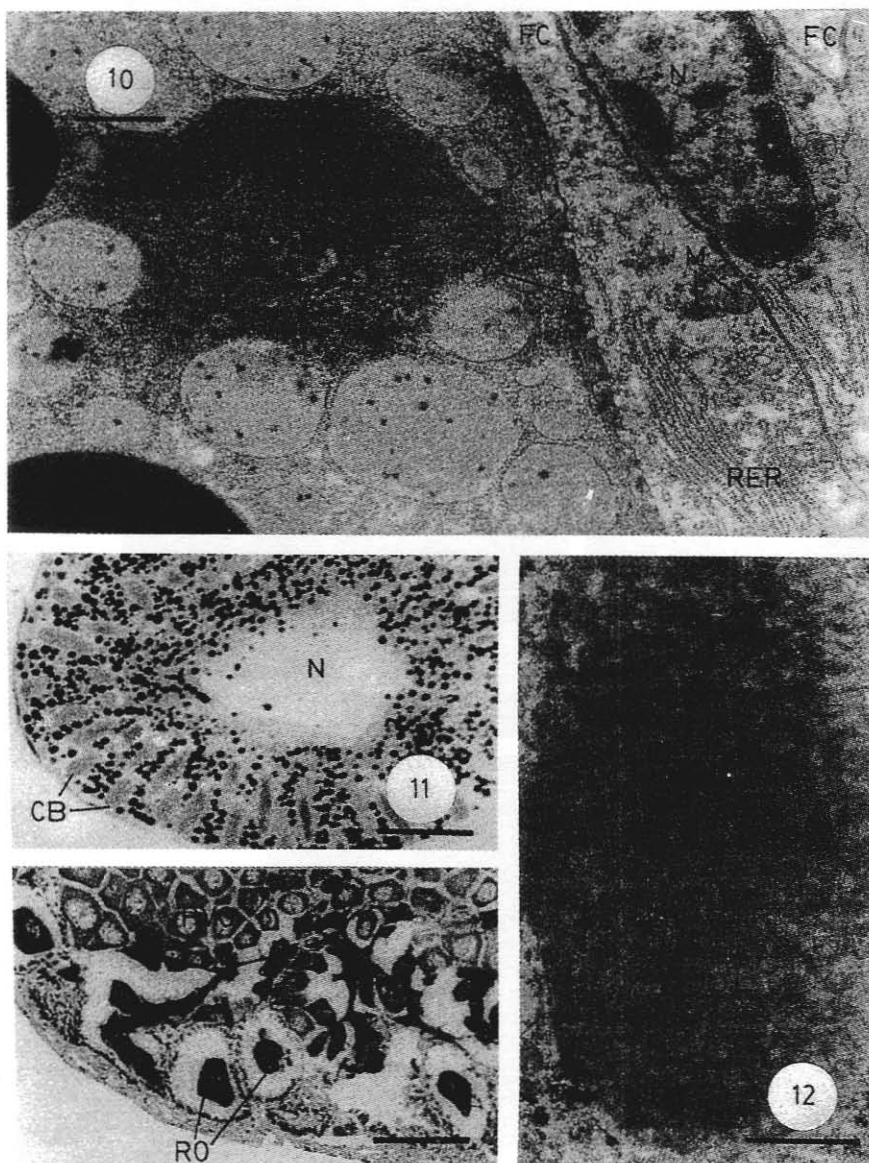


Fig. 10. Electron micrograph of the LVO with oolemma showing micropinocytotic vesicles (PY). The adjacent follicle cells (FC) have hypertrophied nucleus (N), mitochondria (M), and rough endoplasmic reticulum (RER). Bar equals 0.5 μ m.

Fig. 11. Semi-thin section of a vitellogenic oocyte with peripheral cortical bodies (CB) close to the oolemma. N—nucleus. Bar equals 50 μ m.

Fig. 12. Electron micrograph of the cortical body (CB) showing the feathery substructure. Bar equals 0.5 μ m.

Fig. 13. Photomicrograph of the spent ovary with resorbing oocytes (RO) as well as PVO. Hematoxylin and Eosin. Bar equals 50 μ m.

areas of proliferative growth of oocytes (Fig. 13). Empty follicles from which ova have been released had hypertrophied follicle cells. Pycnotic oocytes which have deeply basophilic cytoplasm and nucleus were also observed in conjunction with hypertrophied follicle cells.

The SO were found to be negative to all tests for carbohydrates (Table 3). A striking feature was the intense PAS positivity of the follicle cells, which was removed by acetylation and restored by deacetylation, indicating the presence of 1,2 glycol groups. Follicle cells were also deeply alcianophilic at 0.1 M level denoting large amounts of carboxylated AMP. The cytoplasm of SO showed the presence of acidic proteins and lipids.

DISCUSSION

The process of vitellogenesis in *P. indicus* was completed by the involvement of two main processes. The first was the proliferative phase wherein the primary oogonial cells multiplied to form secondary oogonial cells. The second was the differentiative phase, when PVO accumulate yolk to become MO. The ventro-median germinal zone was observed in all stages of maturity indicating that the ovary is active throughout the reproductive period of the animal. Descriptions of oogonial cells in penaeids as well as other crustaceans are rare. The oogonial cells of *P. indicus* were rich in RNA and protein but poor in carbohydrates, which is similar to that seen in *Balanus amphitrite* (Fyhn and Costlow, 1974).

In *P. indicus*, during PVO and EVO phases, the nucleoli were deeply RNA positive and were observed along the peripheral margin of the nuclear membrane. Further, it is clear from the present study that a considerable amount of the RNA material is extruded out from the nucleus through nuclear pores. Such nuclear emissions are not uncommon in the early vitellogenic oocytes in the autotrophic phase of various crustaceans (Adiyodi and Subramoniam, 1983). The transfer of nuclear material to the ooplasm acts as a prelude to protein synthesis. In addition, the presence of numerous free ribosomes, rough ER, and mitochondria in the cytoplasm indicated that the ovary in *P. indicus* during stage I and II had autotrophic capabilities. The progressive development of an elaborate array of rough ER is found to be an important characteristic of early vitellogenic oocytes of many crustaceans like the mole crab, *Emerita analoga*, true crabs *Pachygrapsus crassipes* and *Cancer* sp., and lobsters *Homarus* and *Panulirus*, which are reported to rely predominantly on autotrophic synthesis (Adiyodi and Subramoniam, 1983; Kessel, 1968).

There was a decided shift in the mode of yolk synthesis to extraoocytic or heterotrophic synthesis when the oocytes advanced to LVO phase. Evidence for this is derived from the presence of pinocytotic vesicles along the oolemmal margin. Further, cell organelles such as ER and mitochondria become scarce in the ooplasm. In the spider crab *Libinia emarginata*, Hinsch and Cone (1969) have described a similar shift during late vitellogenesis due to micropinocytotic absorption. Such a temporal separation of intraoocytic and extraoocytic origin of yolk is also well defined in *Orchestia gamarella* (Zerbib, 1977).

Folliculogenesis in *P. indicus* was initiated during PVO phase and completed by EVO phase. The flattening of the follicle cells with the growth in volume of the oocyte was characteristic of the process. According to Charniaux-Cotton (1985) follicle cells facilitate vitellogenic activity by aiding in the uptake of yolk proteins from external sources. Apparently, in *P. indicus* too, the follicle cells function in a similar manner. Evidence for the protein-synthetic ability of follicle cells was obtained by the presence of rough ER and mitochondria in their cytoplasm. In *P. japonicus*, follicle cells are implicated as the possible cell type responsible for ovarian vitellogenin synthesis (Yano and Chinzei, 1987). Follicle cells of *P. indicus* were exceptionally rich in AMP in all vitellogenic phases. One of the well-documented functions of AMP in vertebrates is that of allowing a ready diffusion of dissolved substances between cells and capillaries (Hohnke and Scheer, 1970). Therefore, it is probable that follicle cells serve and facilitate an exogenous supply of yolk materials.

A striking feature of the mature oocyte in *P. indicus* was the appearance of rod-like cortical bodies in the cortex of the oocytes. Termed variously as marginal bodies (Subrahmanyam, 1965), cortical rods (Duronslet *et al.*, 1975; Tan-Fermin and Pudadera, 1989), peripheral bodies (Rao, 1968; King, 1948), jelly substance (Hudinaga, 1942), and cortical crypts (Yano, 1988), these bodies are unique to the genus *Penaeus*. They do not occur in the oocytes of *Parapenaeopsis stylifera* (Shaikhmahmud and Tembe, 1958). Clark *et al.* (1980) have demonstrated that cortical bodies are responsible for the jelly layer which surrounds the egg during early development. The feather-like substructure observed in the cortical bodies of *P. indicus* obviously aids in the formation of the jelly layer. The cortical bodies were rich in sulphur-containing amino acids and sulphated and carboxylated AMP. According to Meyer (1947), non-sulphated AMP controls the permeation of water (through gel-forming properties) and protects the egg against bacterial attack. The presence of disulphide and sulphydryl bonds may perform a role in aiding cytokinesis in penaeid eggs. In the sea urchin it has been suggested that thiol interaction on proteins may account for the contraction of the equatorial ring during cleavage (Sakai, 1960).

The fully formed yolk in *P. indicus* was observed to be a complex of glycolipoprotein nature. Proteins (tryptophanyl, aromatic and amino end groups) were the first to form in the yolk. Thiol end groups were incorporated into the yolk vesicles subsequently. The carbohydrate moiety of the yolk complex was the next to be formed and lipids the last. Carbohydrate substances were mainly PAS positives and AMP. Sudanophilic lipid bodies were apparent in the ooplasm right from the EVO phase. Only neutral lipids were formed later. In *P. indicus* there was no evidence to suggest an endogenous origin of lipid yolk. The bulk of the lipid is transported to the oocytes from the rich reserves of the hepatopancreas. This organ showed fluctuation in lipid content concomitant with vitellogenesis (Mohamed and Diwan, 1992).

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