Proc. Indian natn. Sci. Acad. **B46** No. 3 pp 287–292 (1980)

Germinal Zone Activity and Oocyte Differentiation in the Marine Crab *Portunus pelagicus*

M MICHAEL ARUL DHAS[1] T SUBRAMONIAN[2] SUDHA VARADARAJAN[2] and P GOVINDARAJULU[1]

(Received 13 September 1979; after revision 21 October 1980)

A cytological study of the various phases of oogenesis has been made in *Portunus pelagicus*. The primary oocytes are derived from a centrally placed germinal zone where no further development of oocytes takes place. Instead, they are pushed to the ovarian lobes where further growth and maturation are completed. The activity of the germinal cord seems to be cyclical as there appears to be no further release of primary oocytes, once oocytes in the ovarian lobes have started growing. The follicle cells seem to originate from the mesodermal cells surrounding the germinal cord, their association with the growing oocytes is intimate.

Key Words: **Follicle cells, Germinal cord, Oocyte, Nucleus, Nucleolus, Yolk globules**

Introduction

Though considerable work has been done on the mode of yolk formation in various Crustacea in recent years (Kessel 1968, Croisille et al. 1974, Lui & O'Connor 1976), not much is known on the generative phase of oogenesis. The present paper reports cytological observations on the manner in which the primary oocytes are formed from the germinative zone and their subsequent differentiation in the vitellarial part of the ovary in a crab *Portunus pelagicus*.

Materials and Methods

Portunus pelagicus is a continuous breeder (Rahaman 1967). Sexually mature female crabs procured from the Royapuram Coast, Madras were immediately opened and the colour of the ovary noted.

The ovaries of *Portunus* were classified into five arbitrary developmental stages based on their colour (Fielder 1964 and Heydorn 1968) and microscopic examination of fresh oocytes. In stage I the ovary is colourless and thread-like. Only clusters

[1]Department of Endocrinology, Post-Graduate Institute of Basic Medical Sciences, Taramani, Madras 600 042
[2]Department of Zoology, University of Madras, Chepauk, Madras 600 005

of small primary oocytes were present. In stage II the ovary is slightly enlarged in size and acquired a slightly yellow colour. Stage III represents a still large yellowish orange coloured ovary whereas in stage IV it is deep orange colour, fully grown and extended into all available space in the haemocoel. The microscopic examination of the fresh oocytes at this stage showed that they were completely filled with yolk materials. The spent ovary represents stage V, the ovary being very thin and almost colourless, containing a few remnant mature oocytes.

For histological studies the ovary was immediately dissected out and small lumps of ovarian tissues were placed in different fixatives such as buffered neutral formaldehyde and Gilson's fluid. Paraffin sections were cut at 7 μ thickness and stained in Mallory's triple stain, Heidenhain's haematoxylin and Ehrlich's haematoxylin.

Results

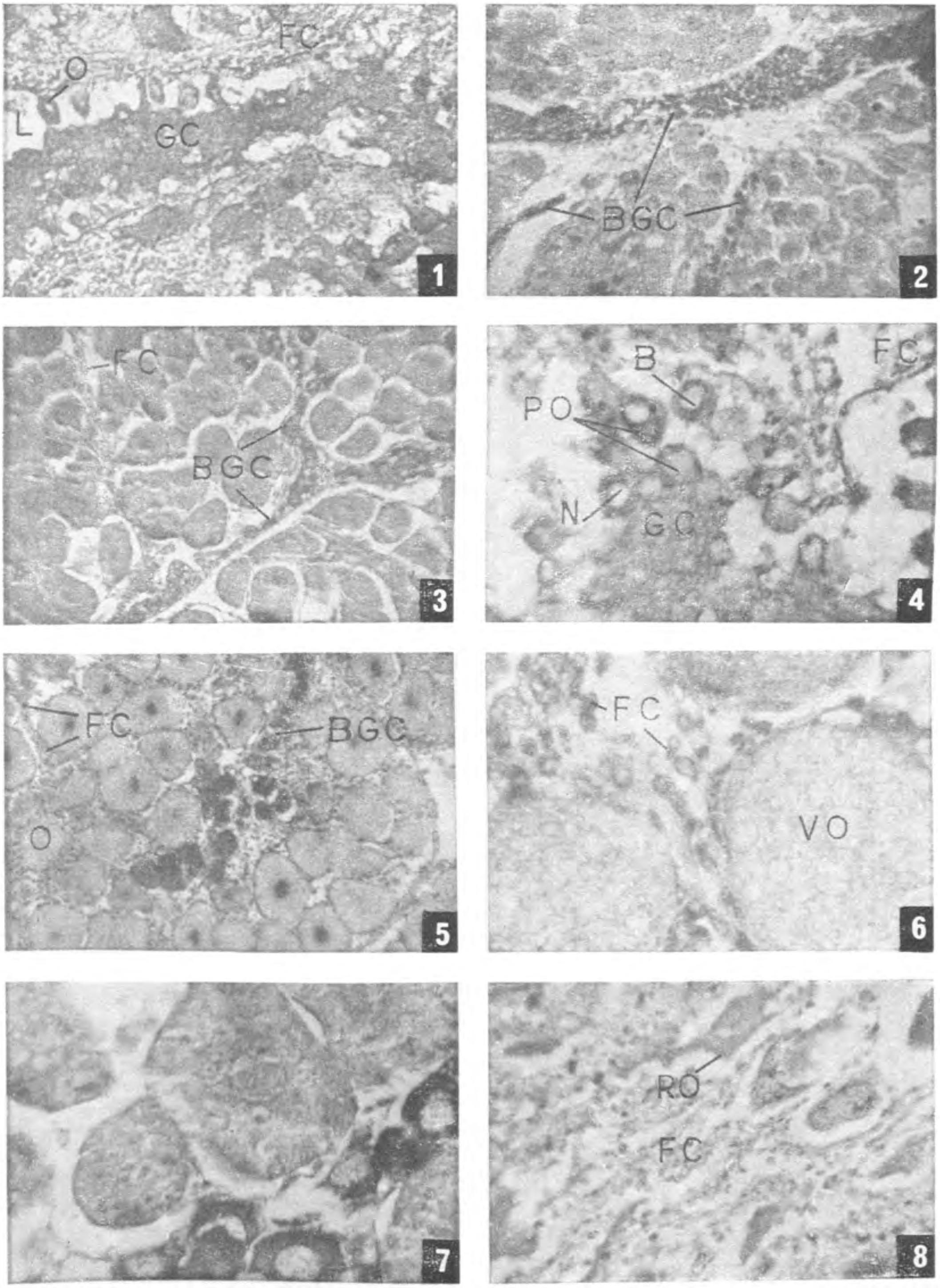
General morphology and histology

In *Portunus pelagicus* the ovary is paired with an 'H' shaped interconnecting bridge in the centre. In the fully grown condition, the anterior limbs are curved sideways, whereas the posterior limbs extend upto the narrow abdomen.

Germinal cord

The two limbs of the ovary and the interconnecting bridge are comprised of small tube-like lobes and each lobe is surrounded by a capsule of thick connective tissue. Connecting all the ovarian lobes is the central shaft of ovarian tissue which runs throughout the length of the paired ovary and is also present in the ovarian interconnection. In stages I and II the central shaft is very prominent. Ovarian sections of these stages show a central shaft consisting of a germinal cord and surrounding this, is a lumen to the outer side of which are attached the ovarian lobes (figure 1). The germinal cord ramify throughout the ovary (figures 2 and 3) and takes intense stain with Ehrlich's haematoxylin. A close examination reveals the presence of darkly staining nuclei in this zone (figure 4). These nuclei seem to have thin layer of cytoplasm around them. The cells are small, spherical and have a prominent spherical nucleus with an unstained cytoplasm. The nucleolus has not been differentiated. These cells are found only in the interior of the germinal cord and may correspond to the oogonial cells described in other decapod crustaceans (Menon 1934, Laulier & Demeusy 1974, Charniaux-Cotton 1975). In the periphery of the germinal cord are seen cells blebbing out (figure 4). These cells, however, are different from those

Figures 1-8 *1*, T.S of Stage I ovary showing the central germinal cord surrounded by a lumen and proliferating oocytes. Haematoxylin (× 55); *2*, T.S of Stage II ovary showing a part of the branched deeply staining germinal cord extending into each follicle. Oocytes have started depositing yolk. Haematoxylin (× 210); *3*, T.S of Stage III ovary showing the germinal cord and branches into ovarian follicle. Haematoxylin Eosin (× 55); *4*, Part of the germinal zone enlarged to indicate the blebbing of the oocytes as well as the oogonial cells inside the germarium. Note the arrangement of follicle cells surrounding the germarium. Haematoxylin (× 475); *5*, T.S of ovarian lobes containing maturing oocytes with immature oocytes locked up in the centre. Haematoxylin (× 210); *6*, T.S of ripe ovary showing oocytes filled with yolk globules. Nuclei no longer visible. Follicle cells surround the oocytes in a single layer except in between follicles. Haematoxylin (× 475); *7*, T.S of Stage IV ovary showing the yolk globules completely filling the ooplasm. Immature, deeply stained oocytes with large nuclei can still be seen between the follicles. Haematoxylin (× 475); *8*, T.S of a resorbing ovary with mis-shapen oocytes and follicle cells in thick strands. Haematoxylin (× 210)


FC
O
L
GC
1
BGC
2
FC
BGC
3
B
PO
N
GC
FC
4
FC
BGC
O
5
FC
VO
6
7
RO
FC
8

referred to above, in that they are larger in size with a deeply stained cytoplasm and an enlarged germinal vesicle. The nucleolus, where evident, is darkly stained but the nucleoplasm has not taken any stain. These cells are pinched off from the periphery of the germinal cord and make their way into the centre of each ovarian lobe. From there, they are pushed towards the periphery until the ovarian lobes are fully packed.

Another interesting observation is the occurrence of immature oocytes in the centre of the ovarian lobe which is filled with growing and maturing oocytes (figure 5). These young oocytes do not show any sign of development and retain the staining property of the primary oocytes at the stage of proliferation from the germinal cord.

Oocytes

The primary oocytes, when detached from the germinal cord, have a nucleus already enlarged into a germinal vesicle with a prominent, eccentric nucleolus (figure 4). The nucleoplasm is also clear. Proliferating oocytes are mainly found in the periphery of the branching germinal cord. Inside the ovarian lobes the larger oocytes occupy the periphery whereas the smaller ones are arranged towards the centre suggesting that the growth of the oocyte takes place immediately after their lodging into the ovary lobes. This growth period is characterised by nuclear changes including the appearance of small basophilic granules in it, in addition to the intensively stained nucleolus. Occasionally these basophilic granular materials are seen dispersed in the periphery of the nucleus.

In ovarian sections of stage III the proliferated oocytes are already inside the ovarioles. But in these two stages, the oocytes have taken only the haematoxylin stain whereas eosinophilia appears in the cytoplasm of the stage III. Sections of stage III reveal the presence of yolk globules beginning to form within oocytes and larger globules are more towards the periphery (figure 6). These globular yolk material slowly fill the inner region of the ooplasm and in the stage IV ovary the yolk globules have completely filled up the ooplasm (figure 7). Interspersed among the yolk globules are small granules. The nucleus at this stage looks diffused and stains homogenously with haematoxylin and the granular structures are no longer discernible. The retention of diffused nucleus in the matured oocytes is a situation found also in *Portunus sanguinolentis* where the meiotic division is completed only after ovulation and fertilization (Ryan 1967). In the spent stage a few unspawned oocytes are seen undergoing degeneration (figure 8).

Follicle cells

Sections of stage I show the differentiation of the follicle cells from the somatic cells encircling the germinative zone (figure 4). These follicle cells seem to slowly stream out to envelop each oocyte in one-cell thickness inside the ovarian lobes (figures 5, 6). The follicle cells are so closely set that cell boundaries are difficult to discern. But they have a deeply staining nucleus and cytoplasm that does not contain any stainable material. The gap between growing oocytes is occupied by follicle cells (figure 5). In the ripe oocytes the follicle cells are seen detached from them facilitating ovulation. In the spent, shrunken ovary the follicle cells remain intact showing no signs of degeneration (figure 8).

Discussion

The results reported in the present paper throw new light on the activity of germinal zone as related to the ovarian development in *Portunus pelagicus*. The position of the germarium in the crab ovary varies widely. It may be peripheral as in amphipods and in the isopod *Lysmata seticudata* (Charniaux-

Cotton 1975), peripheral but confined to a particular region as in *Eupagurus* (*Pagurus*) *bernhardus* (Jackson 1913) and *Clibanarius olivaceous* (Kamalaveni 1947) or in the form of germ nests distributed throughout the ovary as in the land crab *Gecarcinus lateralis* (Weitzman 1966) or central as in *Carcinus maenas* (Laulier & Demeusy 1974). In *Portunus pelagicus* it is in the form of a central shaft of germinal tissue. In the early stage of the ovary-development, this zone is full of basophilic granular masses possibly representing the dividing oogonial cells. In the periphery of this germinal zone, small oocytes with a prominent nucleus are seen budding off into the lumen surrounding the entire length of this germinal cord. It is of interest to note here the observations of Ryan (1967) on *P. sanguinolentis* that the ovarian lumen is in the centre and that the germinal epithelium is placed on the border of the lumen. In *P. pelagicus* the primary oocytes proliferated from the central germinal cord move across the surrounding lumen into the ovarian lobes where they undergo further growth and maturation. Once the oocytes start growing in the lobes of the ovary, the release of new oocytes from the germinal cord ceases suggesting that such a release is cyclical. However, the germinal cord neither shrinks nor is the basophilia of the germinal zone reduced during the maturation of the oocytes recalling a similar observation on the continued activity of germarium in a mature land crab *Gecarcinus lateralis* (Weitzman 1966).

In the ovarian lobes of *P. pelagicus* along with the maturing oocytes, a group of young oocytes also present in the centre showing no sign of development. These young oocytes may be late arrivals and it is possible that they have not received the stimulus for further differentiation and maturation. Hard (1942) also noticed such immature oocytes among the maturing oocytes of *Callinectes sapidus* but believed that their presence was due to the new egg formation from the germinal epithelium. That these young oocytes are not utilized in subsequent gametogenesis is shown by the observation that they are resorbed along with the other unspawned oocytes after ovulation. The implication is that a fresh crop of oocytes formed from the germinal cord for the next gametogenesis.

References

Charniaux-Cotton 1975 L' ovogenese et sa regulation chez les Crustaces superieurs; *Ann. Biol. anim. Biochem. Biophys.* **15** 715–725

Croisille Y, Junera H, Meusy J J and Charniaux-Cotton 1974 The female specific protein (vitellogenic protien) in Crustacea with particular reference to *Orchestia gammarella* (Amphipoda); *Amer. Zool.* **14** 1219–1223

Fielder D R 1964 The spiny lobster, *Jasus lalandei* (H Milne Edwards) in South Australia II Reproduction; *Aust.J.mar. freshw. Res.* **15** 133–144

Hard W L 1942 Ovarian growth and ovulation in the mature blue crab: *Callinectes sapidus* Rathbun; *Chesapeake Biol. Lab.* **46** 3–17

Heydron A E F 1968 The rock lobster of the South African West coast *Jasus lalandei* (H Milne Edwards). II. Population studies behaviour, reproduction, molting, growh and migration; *S. Af. Div. Sea Fish Invest. Rep.* **71** 1–52

Jackson M G 1913 *Eupagurus L.M.B.C. Memoirs* **21**

Kamalaveni S 1947 Biology of *Clibanarius olivaceous*; M. Sc. Thesis, Madras University

Kessel R G 1968 Mechanism of protein yolk synthesis and deposition in crustacean oocytes; *Z. Zelloforsch* **89** 17–38

Laulier M and Demeusy N 1974 Etude histologique du functionnement ovarien au cours d'une maturation de ponte chez le crabe *Carcinus maenas* L. (Crustacea: Decapoda); *Can. Biol. Mar.* **15** 343–350

Lui C W and O'Connor J D 1976 Biosynthesis of lipovitellin by the crustacean ovary II Characterisation of and *in vitro* incorporation of amino acids into the purified subunits; *J. exp. Zool.* **195** 41–52

Menon M K 1934 The oogenesis of *Emerita asiatica*; *J. Madras Univ.* **6** 1–15

Rahaman A A 1967 Reproduction and nutritional cycles of crab *Portunus pelagicus* (Lin.) (Decapoda: Brachyura) of the Madras coast; *Proc. Indian Acad. Sci.* **B65** 76–82

Ryan E P 1967 Structure and function of the reproductive system of the crab *Portunus sanguinolentus* (Herbst.) Brachyura: Portunidae) II The female system; *Proc. Symp. Crustacea, Mar. Biol. Ass. India, Eranakulam, Part II* 522–541

Weitzman M C 1966 Oogenesis in the tropical land-crab *Gecarcinus lateralis* (Freminuile); *Z. Zellforsch* **75** 109–119