Proc. Indian natn. Sci. Acad. **B46** No. 5 pp 645-651 (1980)

Histological Changes during Vitellogenesis in the Ovary of the Hermit Crab *Clibanarius clibanarius*

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(Received 22 *November* 1979*; after revision* 27 *February* 1980*)*

The histology of the maturing ovaries of *Clibanarius clibanarius* showed the imprint of abdominal torsion on the germinal zone. This region initially occupied a large ventral area but became more restricted to the midregion as vitellogenesis advanced. The stress of yolky oocytes on the germinal area accentuated the process further. In extreme cases an internalization of the germinal zone was observed recalling the condition found in adult Brachyura. The association between follicle-cells and oocytes was found to be intimate during yolk formation.

Key Words: Germinal zone, Oocytes, Follicle cells, Vitellogenesis in the *Clibanarius clibanarius*

Introduction

The lodging of the hermit crab in a molluscan shell imposes certain restrictions on its morphology; this is manifest externally by lack of calcification in the abdominal cuticle (Chockalingam 1967), suppression of the right abdominal appendages, and a prehensile uropod (Kaestner 1970). Internally, the right ovary is shorter than the left and the oviducts exhibit different degrees of torsion as exemplified by *Clibanarius misanthropus*, *Eupagurus* (=*Pagurus)* *bernhardus* and *P. prideauxii* (Carayon 1941). These studies do not, however, provide any knowledge as to the effect of abdominal torsion on the intraovarian configuration especially that of the germinal zone whose placement has been the subject of recent investigations in crustaceans (Laulier & Demeusy 1974, Payen 1974).

Among the decapods, the germinal zone may be in the entire periphery and later restrict itself to one side as in Macrura (King 1948), may form the innermost lining of the ovarian wall as in *Menippe mercenaria* (Binford 1913), may exist as a central shaft of germinal tissue as in *Portunus sanguinolentus* (Ryan 1967) and *Pachygrapsus crassipes* (Chiba & Honma 1972) or may form germ-cell-nests as in *Gecarcinus lateralis* (Weitzman 1966). Among Anomura, a ventrally situated germinal zone is apparent in all the hermit crabs investigated (Jackson 1913, Bloch 1935, Carayon 1941, Kamalaveni 1947), but its relative disposition in the two ovaries as a consequence of torsion and yolk-deposition has never been described. For this purpose, a study of the sequential changes undergone by the growing ovaries of *Clibanarius clibanarius* was undertaken.

Materials and Methods

Specimens of *Clibanarius clibanarius* Dana were collected by launches operating in areas where the depth ranged between 10-15 fathoms in the Bay of Bengal off the Madras coast, India. Observations were confined to field-fresh, undamaged females in the intermolt stages. Measurements of oocyte-diameter taken according to Laulier and Demeusy (1974) in conjunction with the ovarian colour, were used to classify the ovaries into four stages. Oocytes with diameter upto 149 μm (early, pale-yellow ovary) represented stage 1; oocytes 150 μm — 379 μm (Yellow-orange or brown-orange ovary) was stage 2, oocytes 380 μm — 540 μm or greater (ripe. deep brown ovary) was stage 3, and the spent ovary with a few resorbing oocytes was stage 4.

For histological studies, ovarian tissue fixed in Bouin's solution or neutral buffered formalin, embedded in paraffin and sectioned at 8 μm were used. For ripe ovaries, thicker sections (12 μm) had to be taken as yolky materials damaged contours in thinner sections. Staining was performed with Mallory's triple stain, Heidenhain's iron haematoxylin or haematoxylin-alcoholic eosin.

Results

Ovarian morphology

The ovaries in *Clibanarius clibanarius* are paired, elongated organs occupying the posterior 2/3 of the abdomen and lie on the hepatopancreas. In stage 1, they are hardly discernible but slightly later, when oocytes measure 80 μm — 149 μm, they are pale yellowish in hue. In stages 2 and 3 they are yellow-orange and deep brown respectively and spread out on the hepatopancreas. Although the ovaries lie side by side they are never interconnected at any stage. The right ovary is slightly shorter than the left. Anteriorly, they are prolonged into two oviducts which open independently on either side at the base of the coxae of the third pereiopods.

Stage 1 ovary

The ovary is thin, pale yellow, transparent and flaccid displaying two regions in transverse section. The first is the germinal zone lying nearer and parallel to the ventral wall containing groups of oogonial cells measuring below 50 μm in diameter (figure 1), in the mitotic stage. Dorsal to these and measuring between 50 μm — 149 μm are cells possessing large nuclei, well-developed chromatin network and a prominent deeply staining, often eccentric nucleolus. The cytoplasm is uniformly granular with no yolk globules. Strands of follicle-cells with deeply staining nuclei criss-cross the ovary at random separating these larger oocytes into groups (figure 1). These strands do not seem to differ from those around the entire ovaries, both staining deeply with haematoxylin. On the dorsal face, however, the ovarian wall is thicker, tending to become more convex. The noteworthy feature here is the identical disposition of the germinal zone in both ovaries [figure 13 (1)].

Stage 2 ovary

The ovarian colour ranges from yellow-orange to brown-orange. This is in a large measure due to the presence of oocytes measuring between 150 μm —379 μm (figures 2, 3 and 4). In sections taken of the paired ovaries together, the germinal zones are already in slightly different regions in both (figure 2). While in the left ovary, the germinal area is still ventral, in the right ovary the position has shifted to a median one between the two ovaries [figures 2 and 13 (2)]. Apart from this, the germinal zone also appears to be restricted to a smaller area than in stage 1 as oocytes steadily increase in diameter owing to yolk-accumulation. Nuclei and nucleoli are still visible in some oocytes (figure 3). The growing oocytes engaged in vitellogenesis move away from the ventral germinal zone to a more dorsal position, distending the ovarian wall on that side. Simultaneously oocytes proliferated anew from the germinal zone take their place.

The follicle-cells at this stage have advanced from their former position adjacent to the germinal zone to surround, in a single layer, the oocytes at the threshold of vitellogenesis (figure 3). Normally rounded with prominent nuclei (figure 5) they flatten themselves to fit the growing oocytes closely. As oocytes increase in size, the follicle cells stretch to their maximum and individual cells are difficult to discern. Mallory's stain, however, distinguishes them from yolky oocytes, the latter staining orange-red as a contrast to the blue staining of follicle-cells and germinal zone. Oocytes gradually become fuchsinophilic while the follicle-cells remain unaltered in their staining properties whether adjacent to the germinal zone or surrounding the oocytes.

Stage 3 ovary

The deep brown, distended ovary contains exclusively oocytes larger than 379 μm in diameter. Nuclei are no longer visible in these oocytes which have for the most part, completed vitellogenesis (figure 6). The yolk-laden oocytes crowd out the germinal area into a restricted one ventrally. In the right ovary this area lies completely along the middle line between the two ovaries displaying maximal torsional effect [13, (3)]. From here, radiating cordons of oocytes fan out, thus displaying in a single transverse section, oocytes of all stages (figure 6). The germinal region staining blue with Mallory's and the yolky oocytes staining yellow-orange found side by side indicate a transition towards fuchsinophily.

That the ventrally restricted germinal area extends as a band throughout the length of the ovary, is evident in a longitudinal section. However, this band is thrown into folds forming a scalloped ventral edge bv the pressure of yolky oocytes (figure 7). In extreme cases, the germinal zone becomes completely surrounded by overhanging vitellogenic oocytes giving it an apparent internal position (figure 8). However, the primary peripheral origin of oocytes is clear in preparations where a string of oocytes intrude into the ovary (figure 9).

Stage 4 ovary

Thin, transparent and flaccid, the ovary representing the spent stage has strands of follicle-cells with a few unspawned oocytes (figure 10). The latter distinguish this stage from the first one. Unspawned oocytes and smaller oocytes that have not been encircled by follicle-cells (figure 11) undergo resorption. Regrouping of follicle-cells and proliferation of future oocytes begin as seen in sections of an ovary soon after oviposition (figure 12).

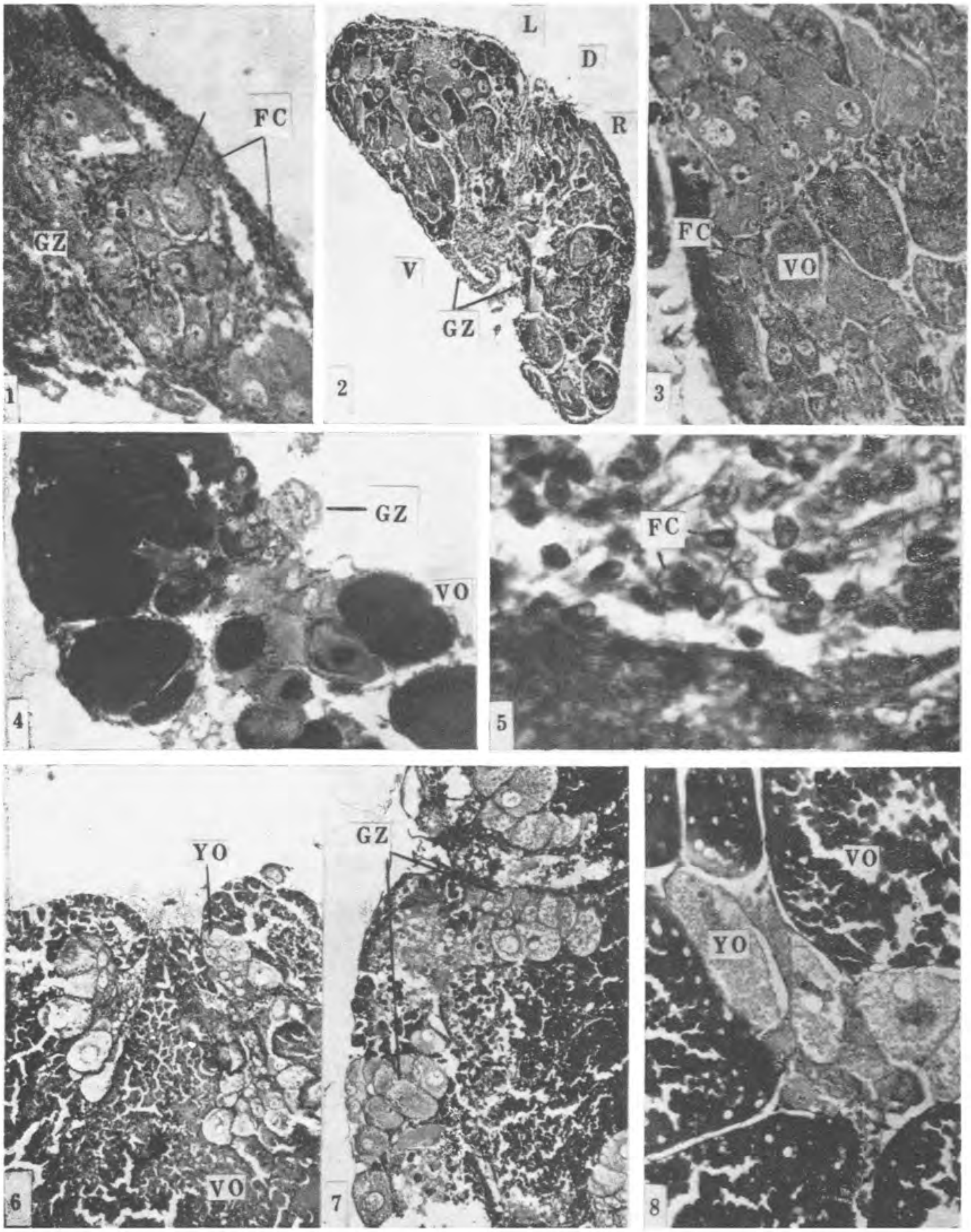
Discussion

Histological studies of the maturing ovaries of *Clibanarius clibanarius* showed the presence in them of two distinct cell-types, the follicle-cells and the oocytes distinguishable from the earliest stage onwards. The stage 1 ovary, dominated by the germinal zone is reminiscent of the post-embryonic ovary at hatching in *Orchestia gammarellus* (Hort-Legrand et al. 1974). In *C. clibanarius*, however, a slightly ventral disposition was already apparent. The first indication of a change in position of the germinal zone was in stage 2 where the right ovary shifted clockwise while the left one remained stationary. This shift in position became more accentuated in the final stage. Further, the germinal zones occupy a much smaller portion of the total ovarian area than initially. This final arrangement alone described by Jackson (1913), Bloch (1935), Carayon (1941) and Kamalaveni (1947) for various hermit crabs led to their general conclusion that the placement of the germinal zone in Anomura was ventral. But the present study indicates that such a placement is a secondary restriction of a previously uniformly disposed germinal area.

Apart from torsion the advent of vitellogenesis in *C. clibanarius* breaks up the early regularity of proliferated oocytes giving rise to their dorsal displacement and biased growth. Thereafter and until the full distention of the ovary, waves of oocytes representing a graded series pass out from the germinal zone to the opposite wall. During this period vitellogenic oocytes weigh down the ventral side of the ovary consequently throwing the germinal zones into folds. Owing to the overwhelming pressure of growing oocytes the germinal zone may even be "imprisoned" among them. This final, extreme, internal position of the germinal zone in *C. clibanarius* finds a close parallel to that found in adult Brachyura. Infact, among decapods such as *Carcinus maenas* Laulier (1974) described the germinal zone in the prepubertal females as peripheral, its internal position found only in the adult, brought about, as in *C. clibanarius* by vitellogenic oocytes. While this is a permanent feature of probably all adult Brachyura, in *C. clibanarius* it persists temporarily, the surrounded and indented germinal zone nearly straightening out after ovulation.

Follicle-cells occur in disorderly strands in the early and spent-stage ovaries; during rapid vitellogenesis, however, they are applied closely to the oocyte periphery. This observation coupled with that of a previous histochemical study

Figures 1-8 *1*, T. S. of stage 1 ovary. Groups of proliferated oocytes criss-crossed by thick strands of follicle-cells. Heidenhain's iron haematoxylin (× 1008); *2*, T. S. of stage 2 ovaries, right and left showing the germinal zone in a restricted ventral area Iron haematoxylin (× 32); *3*, T. S. of stage 2 ovary showing vitellogenic nature of oocytes surrounded by follicle-cells. Iron haematoxylin (× 640); *4*, T. S. of a late stage 2 ovary showing vitellogenic oocytes restricting germinal zone to a very small area ventrally. Note oocytes of graded sizes. Mallory's triple stain (× 128); *5*, T. S. of stage 2 ovary showing the primary spherical follicle-cells. Iron haematoxylin (× 320); *6*, T. S. of stage 3 ovary showing the fanning out of young oocytes in a graded series nearly surrounded by vitellogenic oocytes. Iron haematoxylin (× 80); *7*, L. S. of above. Note the inpushed germinal zone (top centre) shifted from its original ventral position on left. Iron haematoxylin (× 128); *8*, T. S of stage 3 ovary showing darkly stained, yolk-filled oocytes 'imprisoning' young oocytes. Note vacuolated cytoplasm of latter. Iron-haematoxylin (× 320)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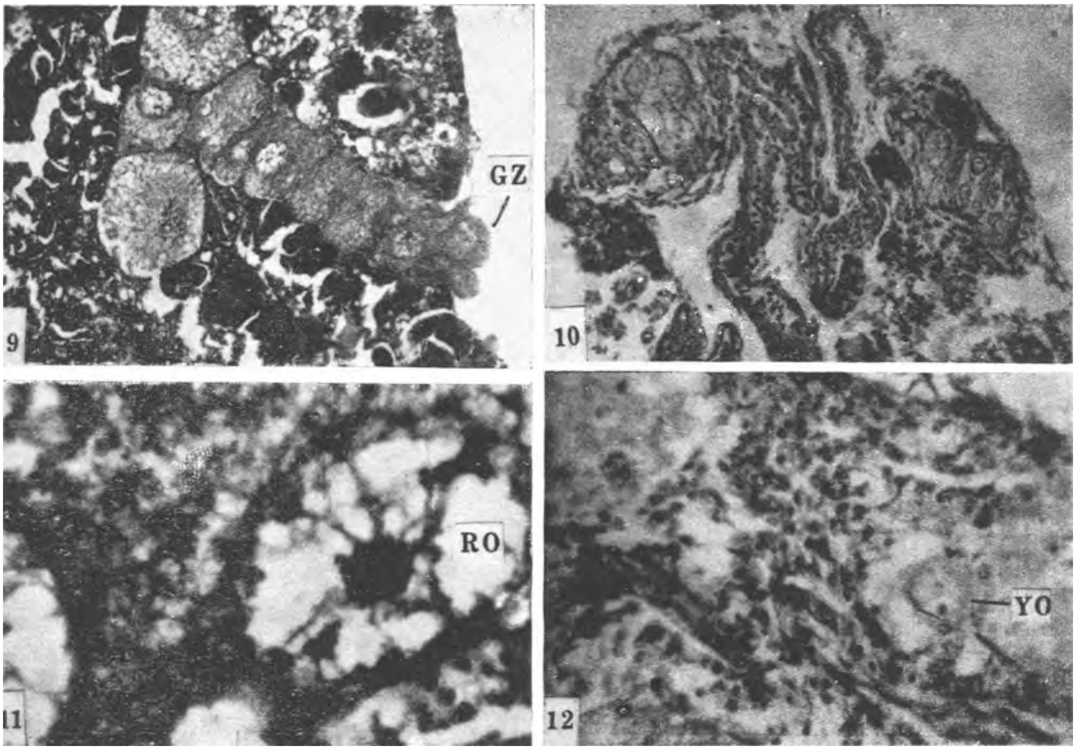

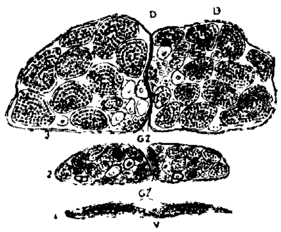

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Figures 9-13 *9*, T. S. of stage 3 ovary. Single strand of intruding oocytes from peripheral germinal zone. Absence of follicle-cells around such oocytes. Iron haematoxylin (× 320); *10*, T. S. of spent ovary. Note persistence of follicle-cells. Iron haematoxylin (× 320); *11*, T. S. of spent ovary showing a young oocyte near germinal zone undergoing resorption. Iron haematoxylin (× 2016); *12*, T. S. of a recuperating ovary soon after oviposition. Note rearranging follicle-cells and next crop of oocytes. Iron haematoxylin (× 1260); *13*, Diagram representing the relative shift of the germinal zone in the two ovaries during oogenesis. (Not drawn to scale). **1**, Stage 1 ovaries with germinal zone more ventral and proliferated oocytes dorsal to it. **2**, Stage 2 ovaries — Note unequal shifts of germinal zones due to torsion in right and left ovaries. **3**, Stage 3 ovaries — Relative shifts of germinal zones before oviposition due to more torsion on the right side and their restricted area due to vitellogenesis

D, Dorsal: **FC**, Follicle-cells; **GZ**, Germinal zone; **L**, Left ovary; **R O**, Right ovary; **V**, Ventral; **VO**, Vitellogenic oocyte; **YO**, Young oocytes revealing their acid mucopolysaccharide nature (Varadarajan & Subramoniam 1980) strengthens the view that follicle-cells may facilitate the passage of macromolecules into the oocyte. Intimate association of follicle-cells and oocytes have also been noted electron-microscopically in *Orchestia gammarella* (Zerbib 1973). Resorption of oocytes lacking follicle-cells suggests that the latter may even regulate vitellogenesis by apportioning available hemolymph proteins to only those oocytes which they encircle.

Acknowledgements

The authors are grateful to Dr K Ramalingam, Professor and Head of the Department of Zoology, Madras University for encouragement and facilities and to Dr K G Adiyodi, Head of the Department of Zoology, Calicut University for constructive criticism of the paper. They thank Mr S M Husaini for translating the French papers.

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