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PROGRESS OF SPERMATOGENESIS THROUGH VARIOUS STAGES OF MOULTING CYCLE IN THE FRESHWATER SHRIMP, *MACROBRACHIUM IDELLA* (HILGENDORF) : A LIGHT MICROSCOPIC STUDY

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The testis of *M. idella* is composed of numerous saccular acini, 115-125μm long and 55-65μm broad. Histological analyses of the testis of animals in different moulting stages reveal that there exists definite correlation between specific stages of the moulting cycle and specific stages of spermatogenesis in *M. idella*.

INTRODUCTION

Spermatogenesis is programmed as a seasonal event in several species of crustaceans, such as *Pontastacus leptodactylus leptodactylus* (Amato & Payen, 1978), *Orconectus nais* (Carpenter & De Roos, 1970), *Gecarcinus steniops* (Philip, 1986). In female natantian decapods, isopods and amphipods, the ovarian growth is programmed in tune with the ecdysial cycles and the accompanying fluctuations in ecdysone titre (Reidenbach, 1971; Adiyodi, 1978; Adiyodi & Subramoniun, 1983). These observations prompted the authors to explore if such a correlation exists between moulting and spermatogenesis in male natantian decapods. The present study was undertaken to clarify this aspect in the freshwater shrimp, *Macrobrachium idella*.

MATERIALS AND METHODS

Sexually mature *M. idella* (5-7 cm long from the tip of the rostrum to the telson) were collected from Olippuramkadavu river, 7 km west of University campus, during two seasons (*i.e.* June- September : monsoon in Calicut and October-December : winter in Calicut). Moulting stages of the shrimps were determined by observing the tip of the uropod under the microscope (Drach, 1939). Animals belonging to various stages of the moulting cycle (intermoult, premoult D1, D2, D3, D4, postmoult, late postmoult) were stored out and grouped. The testes were carefully removed from shrimps belonging to various moulting stages and fixed in Bouin's fixing fluid for histological studies. Paraffin sections 6μm thick were cut and stained with Heidenhain's haematoxylin and 1% alcoholic eosin.

RESULTS

Testis of *M. idella* is composed of numerous saccular acini, 115μm long and 55.65μm broad. There is a general uniformity and considerable synchrony in progress of spermatogenesis throughout the testis of *M. idella*. Table I summarizes the pattern of distribution of mesoderm cells in various phases of spermatogenesis in testicular acini of this species during specific stages in the moulting cycle whereas Table II shows the percentage distribution of various germinal cells in testicular acini at different moulting stages.

Testicular acini of intermoult animals were uniformly filled with mature spermatozoa. To start with the process of spermatogenesis, a number of protogonia appeared along the wall of the acini in the intermoult stage (Fig. 1). The protogonial cells were roughly oval and had irregular contours. Their cytoplasm and nuclei were basophilic. The chromatin was evenly scattered as fine

granules in the nucleoplasm and showed no signs of condensation. The nuclei had an average diameter of 3μm. The cytoplasm formed a thin film, 0.6μm across around the nucleus.

As the animal reached the late intermoult, primary spermatogonia predominated the area adjoining the testicular wall where they were seen to form a single row. Primary spermatogonia were larger than the protogonia and had nucleus about 10μm in diameter. The chromatin of the primary spermatogonia showed a granular appearance; the nuclear membrane of these cells was not significantly basophilic. Cytoplasmic content of the primary spermatogonia was negligible. The mesoderm cells in the testis of *M. idella* were generally arranged towards the periphery of the acini, surrounding either partially or completely the individual protogonia and primary spermatogonia (Fig. 2). Nuclei of the mesoderm cells had roughly oval contours (about 5μm in the long axis across). In some areas, mesoderm cells were found on all the four sides of the protogonia and primary spermatogonia.

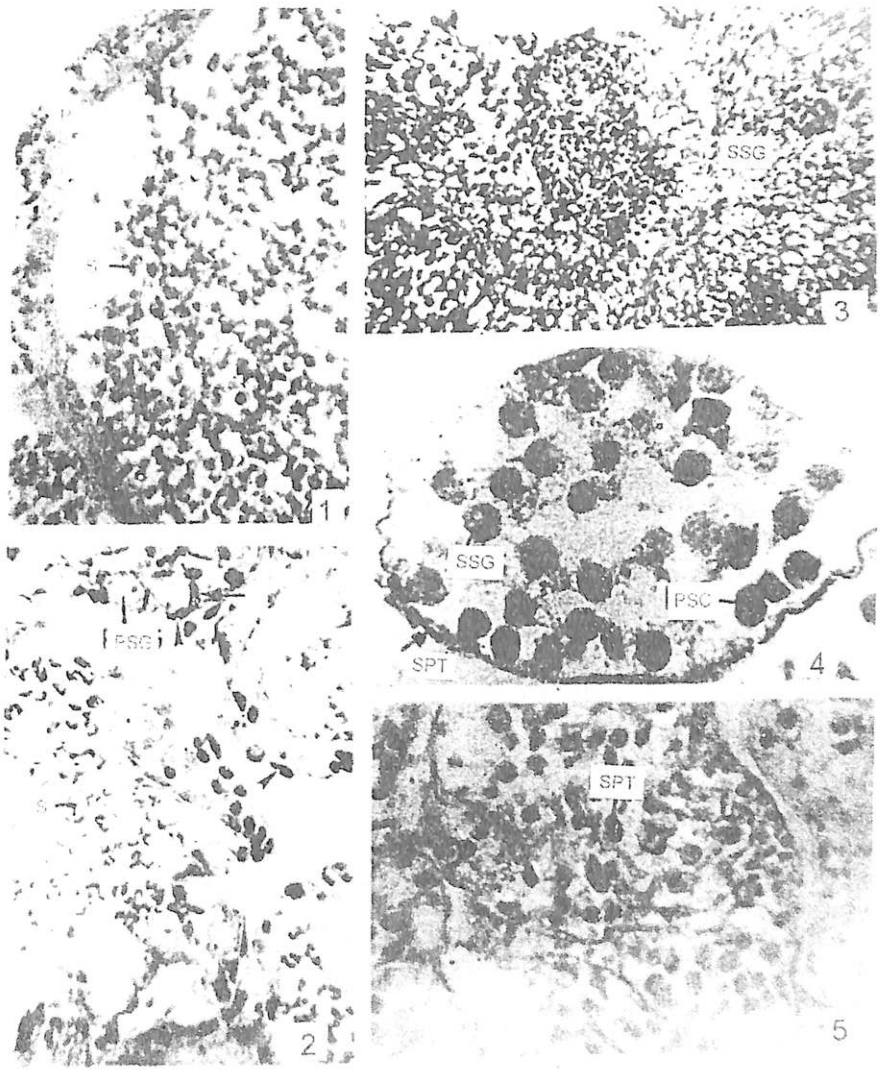
An increase in the number of primary spermatogonia became evident as the animal entered stage D1 of premoult, towards the periphery of the acini. Side by side with this, there appeared to be a fall in sperm content of the acini. It is possible that the spermatozoa were being slowly pushed down towards the vas deferens as the space within the acini was being increasingly occupied by more and more primary spermatogonia.

With the increase in number of primary spermatogonia in the testis of *M. idella*, there occurred a significant change in the pattern of arrangement of the mesoderm cells. Instead of arranging themselves around each primary spermatogonium, the mesoderm cells were now seen to surround each cluster of primary spermatogonia cells.

The testicular acini of *M. idella* in shape D2 were predominantly occupied by secondary spermatogonia (Fig. 3). The latter were smaller than the primary spermatogonia and had spherical nuclei 6.25-7.5μm in diameter and scarce amount of cytoplasm. Almost half the space with in each acinus was now occupied by secondary spermatogonia, leading to a further fall in the number of spermatozoa present within the acinus. Number of mesoderm cells were also declined at this stage. A few mesoderm cells which persisted, had a remarkably thin appearance possessing a length of 6-7.5 μm and a breadth of 1.25μm.

During stage D3 of the premoult, the number of mature spermatozoa present within the testicular acini of *M. idella* declined further, as they were pushed down into the vas deferens. Almost 40% of the area within acinus were now occupied by dividing secondary spermatogonia, while the rest of the space was predominantly occupied by the primary spermatocytes. Although the secondary spermatocytes were not as abundant as primary spermatocytes in testicular acini of *M. idella*, they were nevertheless present in appreciable numbers in all acini. The spermatocytes often appeared eosinophilic and ranged between 2.00 to 3.75μm in diameter. The pattern of distribution of mesoderm cells in testicular acinus of male *M. idella* in stage D3 was comparable to that of shrimps of stage D2. They had a shrunken and elongated appearance.

As the animal entered stage D4, there occurred a perceptible increase in number of the secondary spermatocytes. As much as 75-80% of the area within the acini was filled with both primary and secondary spermatocytes, almost in equal numbers (Fig. 4). The remaining 20-25% of the area within the acinus were occupied by spermatids. The spermatids in their early phase of spermiogenesis had a roughly conical shape, the broader base being rounded and the opposite side showing a slight protuberance. The total length of the spermatids of *M. idella* ranged from 4.25 to 5.00μm.



Figs. 1 - 5. T.S. of testis of *M. idella*. 1. Intermoult testis x 600; 2. Primary spermatogonia x 600; 3. Secondary spermatogonia during D2 of premoult x 200; 4. Primary, secondary spermatocytes and spermatids during D4 of premoult x 600; 5. Postmoult stage showing spermatids x 300 (Formalin, Heidenhain's haematoxylin-eosin) (P=Protogonia; PSG=Primary spermatogonia; S=Spermatozoa; SPT=Spermatids; SSC=Secondary spermatocytes; SSG=Secondary spermatogonia).

Postmoult shrimps had testicular acini nearly fully occupied by spermatids. Secondary spermatocytes were observed only in small numbers (Fig. 5). Eighty to ninety percent of the area within the acini of late postmoult was found to be filled within newly produced spermatozoa. The remaining area, particularly towards the wall of the acini was occupied mostly by spermatids, often in clump like formation. Although mesoderm cells remained peripheral in position in testicular acini, their nuclei were generally larger measuring on an average 7.5μm in length and 2.5μm across. Some cells with streak-like nuclei were also present.

Table I : Percentage distribution of mesoderm cells in testicular acini of *M. idella* (X ± S.E.) various stages of moulting.

Moulting stage	Percentage distribution of mesoderm cells
Intermoult/late intermoult	20.182 ± 2.337
Premoult D1	13.874 ± 2.731
Premoult D2	13.533 ± 1.303
Premoult D3	13.729 ± 2.863
Premoult D4	13.747 ± 1.386
Postmoult	15.128 ± 1.043
Late postmoult	15.742 ± 1.165

Ten replicates were used for each stage.

DISCUSSION

A histological examination of samples of testis taken from animals belonging to different moulting stages clearly reveals that there exists a definite correlation between specific stages of the moulting cycle on one hand and stages of spermatogenesis on the other hand. To our knowledge such a relationship has not so far been reported in any other Malacostraca. In the crayfish, *Ponstacus leptodactylus leptodactylus*, Amato & Payen (1978) have shown that in the annual spermatogenic cycle, specific stages of spermatogenesis occur during specific seasons. In the amphipod, *Orchestia gammarella*, vitellogenesis proceeds parallel to the moulting cycle (Charniaux Cotton, 1981).

In *M. idella*, June to September is the peak moulting-cum-breeding season in females (Narayanan, 1984), and in males (Sreekumar & Adiyodi, 1983). There is a sharp decline in incidence of moulting and percentage of ovigerous females and males from September to January. The values are negligibly low from February to May in the small population of trapped in certain localities and prevented from migrating upstream. In such males both moulting and spermatogenesis cycle are arrested. Our studies have further shown that in such male the androgenic gland is only poorly active. This observation leads us to suspect whether the androgenic hormone has a role in regulating at least some phases of spermatogenesis of *M. idella*. In the marine prawn, *Penaeus indicus* Muthuraman & Adiyodi (1980) have observed a lag in spermatogenesis (especially spermiogenesis) during January-March : the testis did not show any indication of the presence of proliferative phase or signs of maturation division during this period. Testicular acini containing spermatids were common but those with mature sperms were rather few. Though the role played by mesodermal cells in the testicular acini is not clear in *M. idella*, Payen & Amato (1978) found that in the young male crabs and crayfishes, when the androgenic gland becomes hardly perceptible, the gonia differentiate to become spermatogonia under the influence of an androgen inductor elaborated by mesoderm cells.

In *M. idella*, like in the other crabs *Rhithropanopeus harrisii*, *Menippe mercenaria*, *Callinectus sapidus*, *Carcinus maenas*, *Sesarma reticulatam*, *Uca pugilator* and *Ocypode quadrata* and the crayfish *Pontastacus leptodactylus leptodactylus*, the primary spermatogonia in their resting phase are encircled by mesoderm cells (Payen & Amato, 1978). In young crabs and peracarids, it is the androgenic hormone that induces the commencement of subsequent stages of spermatogenesis and the withdrawal of mesoderm cells. Each spermatogonic cycle in *M. idella* is programmed to the moulting cycle, and the mitotic proliferation of the primary spermatogonia begins only with the onset of proecdysis, when the release of the moult-inhibiting hormone from the eyestalks is minimal. This leads us to suspect whether ecdysteroids have a role in

Table II : Percentage distribution of various germinal cells in testicular acini of *M. idella* (S ± S.E.) during different stages of moulting.

Moulting stage	Germinal cells in the acini						
	Protogonia	Primary spermatogonia	Secondary spermatogonia	Primary spermatocytes	Secondary spermatocytes	Spermatids	Spermatozoa
Intermoult/ Late intermoult	7.508±1.095	7.147±1.071	-	-	-	-	85.509±1.671
Premoult							
D1	-	22.947±2.863	-	-	-	-	77.053±2.862
D2	-	-	24.452±1.852	-			75.548±1.852
D3	-	-	40.156±2.242	20.866±3.015	18.789±2.648	-	20.210±5.355
D4	-	-	-	26.004±2.357	32.304±2.724	18.922±0.846	22.700±2.354
Postmoult	-	-	-	-	21.506±3.631	60.755±4.404	17.582±2.041
Late postmoult	-	-	-	-	-	21.418±2.041	78.582±2.041

Ten animals were used for counting during each moulting stage.

spermatogenic activity.

In *M. idella*, a single spermatogenic cycle requires one moulting cycle and its duration has been estimated as 22-25 days. Studies on duration of spermatogenesis of the amphipod, *Orchestia gammarella* by Meusy (1964 & 1972) showed that the total time required for the transformation of spermatogonia into functional spermatozoa is 20 days.

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