

Histology of the mantle and pearl-sac formation in the Indian pearl oyster *Pinctada fucata* (Gould)

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ABSTRACT

The epithelial cells and the associated secretory cells in different regions of the mantle exhibited modifications according to their functions. The pearl-sac formation under laboratory conditions, histology of the mantle and the pearl-sac of *P. fucata* (Gould) are detailed here.

The histology of the mantle and pearl-sac formation in the Japanese pearl oyster *Pinctada martensii* (= *fucata*) has been studied in detail by Ojima (1952), Aoki (1956), Nakahara and Machii (1956), and Machii (1968) and the Australian pearl oyster *Pinctada maxima* by Dix (1973). Ojima (1952) studied the histochemistry of the calcium in the mantle to understand the processes of shell and pearl formation in the pearl oyster *P. martensii*. Regular production of free spherical cultured pearls in the Indian pearl oyster, *Pinctada fucata* began in 1973 (AlagarSwami 1974); however, subsequent work has shown that the quality of the cultured pearls so produced differed considerably in individual oysters (AlagarSwami 1991). The pearl-sac which is responsible for the formation of cultured pearl is derived from the mantle tissue. Hence, some basic studies were carried out on the structure of mantle, its histology, growth of the grafted mantle and formation of pearl-sac in the Indian pearl oyster,

Pinctada fucata (Gould). The results are presented in this paper.

MATERIALS AND METHODS

For the study of the histology of the mantle, fresh healthy pearl oysters (shell height 45-55 mm) collected from the farm of CMFRI at Tuticorin were used. Pearl-sac formation was studied on 180 pearl oysters of the above size range, implanted with the grafted tissue and wax nuclei of 3, 4 and 5 mm diameter. The implanted oysters were maintained in the laboratory with the water temperature ranging from 29° to 30°C for 30 days on a diet of mixed algae and cultured diatoms. Two oysters were cut open every day and the gonad with nucleus *in situ* preserved in neutral formalin. The procedure was continued for 30 days.

To study the histology of the nacreous pearl-sac formation, the gonad portion of the oyster with the pearl inside were carefully fixed in neutral formalin and Bouin's. After 6 hr, the pearls were carefully removed without damaging the pearl-sac and connective tissues of the gonad. The gonad was refixed in fresh fixative for another 18 hr. The tissues were dehydrated in isopropanol and ethyl alcohol, cleared in xylol and benzene separately, and

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embedded in wax with a solidification point at 56°—58°C. Sections, 6-7 µm thick, were cut at room temperature (25°-28°C) and stained with Ehrlich's haematoxylin, cosin and mounted in DPX.

For mantle histological studies, the tissues from ventral fold and isthmus of marginal mantle, pallial mantle and central mantle near the gill attachment were processed as above and sections taken.

RESULTS

Mantle Histology

The mantle of pearl oyster consists of two identical lobes, right and left, united dorsally along the hingeline to form the mantle isthmus. The mantle lobe is divided into (i) marginal zone, (ii) pallial zone and (iii) central zone (Velayudhan and Gandhi 1987).

Marginal zone. The free margin of the mantle lobe was thick, pigmented and fringed with tentacles. The marginal mantle was composed of the inner, middle and outer folds (Fig. 1A). The outer and middle folds were separated by the periostracal groove. Morphologically the folds were similar but differed in the function.

INNER FOLD (Fig. 1 A). The inner fold was larger than the other two folds of the marginal mantle. It was covered with a single layer of ciliated epithelium (25 µm high) with basal nuclei. The inner portion of the mantle showed prominent pigmentation (PE). A strong band of longitudinal and transverse pigmented muscles (MS) was present below the epithelial layer. Acidophilic secretory cells (AS) measuring 3—5 µm were less while the wandering cells (WS) were more in the sub-epithelial cells. In the epithelial cells of the outer margin of the inner fold and also in the sub-epithelium acidophilic and basophilic cells were highly concentrated towards the edge of the fold.

MIDDLE FOLD (Fig. 1 C, D, E and Fig. 2 C). The inner margin of middle fold was con-

stituted like the inner margin of inner fold, but in the latter the epithelium was ciliated (20 µm) and columnar (CC) in nature with pigmentation. Wandering acidophilic mucous cells (BSS) were comparatively more at the tip of the middle fold. Granulated acidophilic cells were also present. The ciliated columnar epithelium (25 µm) of the inner fold further elongated near the periostracal groove (PG) and reached the size of 35 µm, while at other places, they were cuboidal, brush-bordered (7-15 µm) and non-ciliated.

OUTER FOLD (Fig 1 D, E and Fig. 2A). The outer surface of the fold was covered with specialized cells (NE). Elongated (30-35 µm) stratified columnar epithelial cells (ST) occurred close to periostracal groove (PG) on the inner surface of the fold. Non-ciliated and non-pigmented low columnar epithelium (10-15 µm high) containing basophilic cytoplasm were present on major part of the outer fold, becoming elongated (10-20 µm) towards the tip. Basophilic cells occurred more on the sub-epithelial layer near the periostracal groove. Mucous cells were more on the inner margin of the fold, and the acidophilic cells towards the tip.

MANTLE ISTHMUS (Fig 1 I, F). Mantle isthmus or dorsal mantle consisted of non-ciliated columnar epithelium (30-45 µm) with muscle fibres scattered below. On the dorsal side a few secretory cells (IS) were present, and can be observed when stained in Ehrlich's haematoxylin eosin. Sub-epithelial secretory cells were totally "absent".

PALLIAL ZONE (Fig. 2 A and B). The outer epithelial cells were nonciliated and small (4-8 µm) than the ciliated inner epithelial cells (10-30 µm). In between was the muscular connective tissue. In the outer epithelial cells of the pallial mantle and sub-epithelial layers were present large vacuolized/porous secretory cells (SC). The basophilic mucous cells and granulated

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ilic non-eiialed columnar epithelium (IS) of the mantle isthmus.

acidophilic secretory cells (SC) were encountered both in the outer and inner epithelial cells of the pallial mantle.

CENTRAL ZONE (Fig 1 B; Fig 2 B). The outer (shell) side of the central mantle was lined with low columnar epithelium (10-15 μ m). The epithelial layer contained acidophilic secretory cells (AS) and basophilic mucous cells (MU). Histologically, the secretory cells (SC) of inner epithelium of the central mantle looked similar to those of the epithelial cells of the inner pallial mantle.

PEARL-SAC FORMATION AND ITS HISTOLOGY (Fig. 2. D, E, F). Formation of pearl-sac was observed in oysters within 3-7 days after implantation in case of 3 mm nuclei, 4-10 days in the case of 4 mm nuclei and 6-12 days in the case of 5 mm nuclei, when the water temperature in the laboratory was maintained at 29°-30°C. Graft mantle of 2 mm² was used. The nuclei were made of paraffin-wax and implanted in the visceral tissue between the base of the foot and intestine. A thin film of nacreous coating was found deposited on the nucleus within 18 days on a 4 mm wax nucleus.

Histological studies of the nacreous pearl-sac showed the presence of more cuboidal, flattened non-nucleated epithelial cells (CP) along with large secretory cells (4-6 μ m). The cells were similar to the cells of the muscular tissues of the gonad. The haemocytes of the gonad tissue extended into the pearl-sac epithelium. The nucleus was in the centre and occupied much of the cell space. The secretory cells were scattered within and beneath the pearl-sac epithelium. The acidophilic secretory cells were more with large granules (AS) and the basophilic mucous cells were few and these two types of secretory cells were located within and beneath the pearl-sac epithelium.

In case of periostracal pearl-sacs (which were produced with the wax nuclei in the laboratory) tall, ciliated columnar epithelial cells (CE) (30-35 μ m) were well distributed. The cells had basal nuclei and small granules. In some parts of the sac projections of 10-15 μ m were seen. Congregations of cells resembling haemocytes were also present in some areas of the epithelium. Basophilic mucous cells with granular inclusions were common. Acidophilic cells with large secretory granules were present in some parts of the pearl-sac.

DISCUSSION

The regional as well as functional differentiation of the mantle is marked in *Pinctada fucata*. The marginal mantle consisted of 3 folds, inner, middle and outer folds. The inner fold was muscular, the middle sensory and the outer shell fold secretory in function (Dix 1973). The specialized, elongated columnar epithelial cells, occurring close to the periostracal groove, may be the ones which secrete the periostracum in *Pinctada fucata*. A similar type of stratified columnar cell has been recorded in *P. maxima* (Dix 1973). The non-ciliated non-pigmented low columnar epithelial cell, scattered on the other parts of the outer fold suggested a different function.

The inner fold was larger than the middle and outer folds. It had strong longitudinal and transverse pigmented muscles. Wandering cells, which might be sensory in function, were distributed in the sub-epithelial cells of the inner and middle folds. Apart from the wandering cells, the presence of a large number of acidophilic cells in the middle fold suggested an additional function for this fold, probably sensory.

The outer epithelial cells of the pallial and the central mantle were small and non-

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porous granulated acidophilic secretory cells were
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epithelial layers of both pallial and central mantle. These and their proximity to the shell suggested their secretory function, particularly of the inner nacreous layer. The epithelial cells of the marginal mantle along with the inner surface area were ciliated with secretory cells suggesting a different function for these cells.

The epithelial cells of the nacreous pearl-sac differed in size and shape from that of the periostracal pearl-sac. Some similarity was seen between the periostracal pearl-sac and to that of the outer epithelial cells of the pallial and central mantle regions. The presence of haemocytes in the epithelium and sub-epithelium, the large acidophilic secretory cells with granules, and the few number of the basophilic mucous cells were the other similarities. The tall ciliated columnar epithelial cells with basal nuclei and small granules, the irregularly arranged cells with projections and the presence of basophilic mucous cells with granular inclusions are the characteristic features of the periostracal pearl-sac. To a certain extent, these characters are common in epithelial cells found in the periostracal groove, the function of which is to secrete the periostracum of the shell. The presence of both types of secretory cells in the mantle regions and in the pearl-sacs indicate their function, i.e. to participate in the secretion of colcholin. Ojima (1952) is of the opinion that the middle fold of mantle is the main portion for secretion of colcholin. The presence of secretory cells in other parts of the mantle indicates that the shell formation is not restricted to the middle fold. The mucus takes a significant part in the secretion and

deposition of conchiolin.

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