CHAPTER 4

STUDIES ON THE MORPHOLOGY, ANATOMY, HISTOLOGY OF MANTLE AND PEARL-SAC OF PEARLOYSTER
Pinctada fucata (GOULD)
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4.1 INTRODUCTION

Jameson (1901) stated that the identity and distribution of pearl oyster species of the genus Pinctada were difficult to separate from one another by a hard and fast line owing to the absence of well-marked diagnostic characters and the extraordinary geographical and ecotypical variations. However, to achieve satisfactory results in the production of cultured pearls, it is absolutely essential to have a precise knowledge of the morphology, and the anatomy of the animal. Examination of the morphology and structure of the foot, gonad, viscera, and the gonad show that they play a very important role in the production of a pearl and that the gonad has got another vital role in the nourishment of the pearl sac.

Herdman (1904) has given a detailed account of the anatomy of Indian pearl oyster Pinctada vulgaris (= P. fucata). Hynd (1955) and Kuwatani (1965 a, b) also contributed to our understanding of the pearl oysters of Australian and Japanese waters, in addition to throwing light on the functional role of digestive and reproductive organs.

Many workers explained the theory of pearl formation as the defence reaction of the system when a foreign particle gets into the shell causing irritation. In due course, the epithelial cells of the mantle form a sac and secrete pearly coating over this particle, which forms the pearl. The same principle was adopted in the production of cultured pearl by implanting a piece of the mantle along with a shell bead nucleus made out of a molluscan shell. The histology of mantle and pearl-sac formation in Japanese pearl oyster Pinctada martensii (= fucata) has been studied in detail by Ojima (1952), Aoki (1956), Nakahara and Machii (1956), and Machii (1968) that of the Australian pearl oyster Pinctada maxima by Dix (1973); Zahab et al. (1992) and Comps et al. (2000) that of Pinctada margaritifera, Garcia-Gasca et al. (1994) that of
Pinctada mazatlanica. Awaji and Suzuki (1995) and Wada (1996) studied the histology and process of pearl formation in *Pinctada fucata martensii*. 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 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) to analyse the aspects that would help to increase the percentage of survival and the quality of pearl.
4.2 MATERIALS AND METHODS

4.2.1 Morphology and Anatomy

For the study of the anatomy and morphology of the pearl oyster, fresh healthy *P. fucata* (DVM 57 ± 1 mm) collected from the farm of CMFRI at Tuticorin was used. Thirty animals were narcotized using menthol and preserved in neutral formalin. The animals were dissected out aseptically and each organ was carefully examined and thoroughly studied. In some cases live animals were also examined. To understand the course of the arterial system, Alizarin Red stain was injected through the auricles to locate the arteries and veins before dissection. The nervous system, the animals were treated in dilute picric acid solution for ½ hour before dissecting and tracing the nerve innervations.

4.3 HISTOLOGY

4.3.1 The Mantle

Fresh healthy *P. fucata* (DVM 57 ± 1 mm) collected from the farm of CMFRI at Tuticorin were used for the study. The mantle, tissues from ventral fold, isthmus of mantle, pallial mantle and central mantle near the gill attachment were cut out, fixed in neutral formalin, treated, sectioned 6-7 μm thick, stained, processed and mounted following the histological procedures explained by Weesner (1960).

4.3.1.2 Pearl - sac formation

4.3.1.2.1 Preparation of wax nuclei

The wax was put in 250 ml glass beaker and kept in a bath at 58° C for melting. The cooled wax was balled into 3, 4 and 5 mm diameter spherical nuclei and floated in cooled filtered seawater before implantation. On cooling the radius of the wax ball showed minor variations. 3 mm, 4 mm and 5 mm nuclei were 3.12 ± 0.11, 4.09 ± 0.1 and 5.09 ± 0.06 mm respectively. The variations were as follows 3 mm - 3.12 ± 0.11 mm to 4.09 ± 0.10 and 5mm - 5.09 ± 0.06.
Pearl-sac formation was studied in 480 pearl oysters (Plate 4.10B) graft tissue of dimension 2 ± 0.05 mm x 2 ± 0.05 mm (Plate 4.11) and wax nuclei of 3, 4 and 5 mm diameter (Plate 4.12A). The implantation of pearl oysters was done by using the surgical instruments designed and fabricated in India (Plate 4.10). The implanted oysters were maintained in the laboratory in 6 numbers of 200 l FRP tanks each holding @ 100 l sea water at water temperature ranging from 29± 1° C, pH 8.2 salinity 35 ppt. The animals were fed on a diet of mixed algae and cultured diatoms at a feeding intensity of 2 l oyster day for each oyster till the termination of the experiment. Two oysters were cut open from each lot every day and the gonad with wax nucleus in situ was preserved in neutral formalin. Six hours after fixation, the mantle, gill and foot of the animals were carefully cut and removed. After ascertaining the “A” position of the gonad (Plate 4.16B) where in the nucleus with the pearl sac is situated, it was cut carefully using a surgical blade and preserved in neutral formalin for 18 hrs. The nucleus and pearl sac were then sectioned 6-7 μm thick stained and mounted as per the procedures explained by Weesner (1960).

To study the progress of the graft tissue to form pearl sac over the wax nucleus the same procedure was followed. The change in the structure and size of the graft tissue was observed and recorded. A set of 30 pearl oysters were implanted with 4 mm (4 ± 0.02) shell bead nuclei and 2 ± 0.05 mm x 2 ± 0.05 mm (as in the case of wax nucleus) was kept in the farm for 4 months for studying the formation of nacreous and abnormal pearls. For the abnormal pearl formation mantle graft tissue was taken out of the marginal zone of the mantle.

To study the histology of the nacreous pearl-sac, abnormal pearl sac, the gonad portion of the oyster with the quality and abnormal pearl inside were carefully fixed in neutral formalin and Bouin's fixative. After 6 hrs, the pearls were carefully removed without damaging the pearl-sac and connective tissues of the gonad. The gonads were refixed in fresh fixative for another 18 hrs. The sections were cut as above and processed as per protocols mentioned before.
In all the cases, the slides were examined and photomicrographs were taken with the help of Erma scope and computerized prints were taken with the help of Zeiss binocular microscope with digital camera attachment under different magnifications from 5 x to 100 x (oil immersion objective). For morphological studies the detailed drawings of the dissected organs were drawn and for anatomical studies prepared slides were examined, photographed and printed as mentioned above.
4.4 RESULTS

4.4.1 Morphology and Anatomy of Pearl Oysters

The details of shell characters have already been explained in detail in Chapter II

4.4.1.1 Foot

The foot is a highly mobile, tongue shaped organ capable of great elongation and contraction (Plate 4.1B). It arises from the anterior region of the visceral mass midway between the mouth and the intestinal loop and the anterior branchie flanking it on either side. The major part of the foot is composed of network of fibres running in various directions ensuring a wide range of contractibility. The foot is provided with blood spaces and is innervated by nerve fibres making the organ a highly sensitive and active one.

4.4.1.2 Byssus Gland

The byssus gland (Plate 4.2B) is lodged at the proximate end of the foot ventrally. The byssal gland lodges the common root of a bundle of stout laterally compressed bronze green fibres, the byssal threads. Each fibre of the byssus anchors the pearl oyster to rocks and other objects by means of a discoid attachment at the distal extremity. The anterior edge of the byssal gland passes into the pedal groove extending medially along the whole of the remaining length of the ventral surface of the foot. The byssus threads are secreted by this gland and attachment is effected by the highly motile foot.

4.4.1.3 Muscular System

The pearl oyster is monomyarian, possessing only the posterior adductor the largest and the most important muscle in the body.

4.4.1.3.1 Adductor muscle

The adductor muscle (Plate 4.2A) stretches transversely across the body form the valve to valve. It is a massive wedge shaped bundle. The narrow end points upwards and lies immediately behind the ventricle of the heart. The terminal part of the rectum runs in the middle line along the
posterior surface. Two distinct regions of the muscles are obvious; one a narrow tendonous strip made up of white glistening fibres forming the posterior border and the other, a broad and massive semi-translucent fibres occupying the remainder of the mass. The power exerted by the contraction of this muscle is considerable, the rapid action of which resemble ratchet mechanism.

4.4.1.3.2 The Retractor

The retractor of the foot are a pair of symmetrically disposed muscles lying in the horizontal plane of the body (Plate 4.2B). The V-shaped muscles originate from the byssal gland. The ends of this muscle are attached to the right and left valves without making a separate scar on the nacre.

4.4.1.3.3 Levators

The levators of the foot are four, two anterior and two posterior (Plate 4.1B). Each of the anterior pair has its insertion at the apex of the umbonal recess of its respective valve pressing vertically downwards on either side of the mouth spreading laterally, fan-like as they go. The left anterior levator is strong and by contraction of the strong cord of fibres, the foot is drawn over the left side of the valve, which is convex and more spacious. The posterior levators are two short insignificant bundles, which originate high upon the anterior levator, exactly on level with the mouth passing through the visceral mass to be attached to the valves behind the anterior levator scar. The contraction of the anterior levator causes the foot to be retracted and dorsally raised. The intrinsic muscles of the foot are diffused forming a muscular enveloping sheath in the foot, with ill-defined muscle bundles passing from side to side, providing a framework wherein the tubules of the digestive glands ramify. The branchial muscles cause the shortening of the gills and withdrawal of their posterior extremities. They run within each ctenidial axis (Plate 4.2B) from end to end, close to the dorsal edge.

4.4.1.3.4 Pallial muscles

The pallial muscles (Plate 4.1B) are all retractor, and together constitute the orbicular muscle of the mantle. They are a series of fan-shaped
muscles radiating towards the mantle edge from a number of insertion centres (15-18) (Plate 4.1B) of various sizes arranged semi-circularly. Together these form the well marked pallial line scars on the shell. With the exception of heart and indistinct striation on the larger portion of the adductor, the muscle fibres are non-striped.

4.4.1.4 Digestive System

The oesophagus (Plate 4.2A), stomach (Plate 4.2A) and the greater portion of the intestine lie within the viscero-pedal mass. Two horizontal lips conceal the aperture of the mouth. The labial palps (Plate 4.2A) are smooth on the surface, turned away from the mouth and grooved on the opposed faces enclosing the mouth aperture. The mouth (Plate 4.2A) is a large, slit like depression placed transversely between the anterior levator muscles of the foot. The cavity contracts inwards to the narrow width of the short conducting tube, the so called oesophagus, which is straight, dorso-ventrally compressed and ciliated (Plate 4.2A). The hinder end opens into the anterior end of the stomach, which is an organ of surprising elaboration. Folds and depressions diversify the walls and floor of the stomach and break them into definite areas. The tissues consist largely of greenish brown masses often termed as liver (Plate 4.2A) termed as digestive diverticula (Plate 4.2IA). Dense clusters of secreting alveoli open into ductules and these larger ducts lead into the cavity of stomach. The most conspicuous portion of the stomach is a slightly projecting vertical fold arising from the posterior wall marking out the cardiac stomach into a right and left chamber. This fold disappears towards the roof where it is smooth and unbroken, except for a well marked pit. The wide bipartite opening into the intestine and intestinal caecum marks the hinder end of the pyloric chamber. A gelatinous rod flattened and oblique occupies the sub-central position anterior to where the postero-ventral fold disappears midway along the floor. To the right of this area of the dendritie plate is a ridge with a furrow running up to the anterolateral bile duct. A deep rugose-sub-oesophageal pit is well marked, anterior to the dendritic plate and high upon the right lateral wall. The posterolateral furrow leads from the posterolateral duct towards the intestinal aperture. On the left side, a short anterolateral fold lies between the pre-intestinal
depressions of suboesophageal pit.

There are eleven terminal ducts opening into the intestine (a) anterolateral duct, (b) posterolateral duct opening to the posterior third stomach, (c) the postero-ventral duct, (d) three subventral ducts, (e) two pre-intestinal ducts opening within preintestinal depression and (f) three small suboesophageal ducts below the oesophageal aperture.

The head of crystalline style (Plate 4.2A) projects out of the sac where it is formed and across the cavity of the stomach where it bears against an irregular area of cuticle bearing a projecting tooth, known as the gastric shield. This area is not ciliated.

The intestine can be divided into three sections of approximately equal length such as the descending, the ascending portion and the rectum (Plate 4.2 A). The first portion passes ventrally through the posterior part of the visceral mass. Then it passes behind the base of the byssal gland and between the two pedal retractor muscles wherefrom it changes its direction curving forwards and downwards to the visceral mass passing on as ascending branch. A longitudinal fold projects inwards from the anterior and one from the posterior wall of the descending intestine. The apices of the two folds are so close together at the lower third so as to form two distinct tubes. The larger cavity is completely filled with a clear gelatinous solid cylinder, the crystalline style (Plate 4.2A). The narrow tube on right side is the true intestine, the wider left being the sheath of the crystalline style, which is imperfectly separated from the anterior portion of intestine, with which it communicates by a longitudinal cleft. The upper end of the style certainly projects into the stomach. The valvular folding of the intestinal ridge gives entrance to the ascending intestine which curves back-wards along the base of the visceral mass to the left of the descending intestine). The ascending portion crosses to the right at the posterior extremity of the ventral surface of the visceral mass where the two intestinal divisions intersect. The intestinal loop thus formed is the visceral loop. From the point of intersection the ascending intestine turns sharply upwards, running parallel with and closely adjacent to the upper part of the descending portion. The portion of the
intestine forming the second limb of the visceral loop is continued into it as a somewhat undulating ridge disappearing midway. At the point where this diversion of the intestine assumes a dual course, an increase takes place in diameter, side by side with the appearance of a long longitudinal fold-typhlosole, projecting from the anterior wall, curving over to the posterior side of the tube, and then running vertically upward without further change of course. Longitudinal furrows channel the surface. As it approaches the level of the floor of the stomach, the typhlosole thins down rapidly to a low ridge, and the intestine itself then curves posteriorly in the direction of the heart. This change in direction and thinning of typhlosole marks the beginning of rectum.

The rectum runs parallel posterior through the upper part of the pericardium. Beyond this it curves ventrally and passes round the posterior aspect of adductor muscle in the median line ending in an erectile ear like process, the anus, situated opposite the exhalent orifice of the mantle. The anal process is comparatively larger and slightly curved. Anal pappila is trifold (Plate 4.2A). It stands out at right angle to the last section of the rectum, and the tip is directed posteriorly. The anal aperture is situated at the base on the ventral aspect.

4.4.1.5 Respiratory System

The gills (Plate 4.2B) consist of four crescent shaped plates, two half gills on each side which hang down from the roof of the mantle cavity like book leaves. They represent a series of ciliated sieves the whole constituting a feeding surface of utmost efficiency. Two rows of long delicate branchial filaments (Plate 4.2B) are inserted at right angles along the whole length of the axis or vascular base which extends from the ventral border of the palps anteriorly curving round ventrally and posterior to a point opposite the anus with its convexity first forwards and then downwards. Where they terminate the mantle lobes of the two sides are briefly united by way of the inner mantle folds thus dividing the mantle cavity into a large inhalent chamber containing the gills and a much smaller exhalent chamber. Water enters by the one and leaves by the other. The outwardly directed parallel filaments of each series are folded upon themselves, so that they are V shaped, the folding being in
such a way that external filaments turn outwards and internal inwards. Consequently each branchial plate furrow of the double filaments consists of two lamellae, the direct and the reflected, which enclose narrow interlamellar space. The common base of each ctenidium is a vascular attached ridge reaching from the anterior end of gills. Hollow outgrowths, inter-lamellar junctions, containing branches from the afferent vessels (AV) (Plate 4.2) convey blood from the axial trunk to the base of reflected lamellae. The blood enters certain of the individual filaments, flows outwards to the free margin, passing over to the direct filaments returning inwards to the branchial or ctenidial axis (Plate 4.2B) where it joins the different vessel by openings along each side. Neighboring filaments are joined by continuous organic union mainly at the lower and the upper ends of the reflected filaments, where there are longitudinally running blood vessels. Elsewhere the filaments are joined chiefly by the interlocking stiff cilia of the large ciliated discs, which occur at intervals, throughout their length. The normal function of the ordinary cilia on the branchiae is to create a current of water, which enters the pallial chamber and passes over and through the branchial lamellae so as to purify the blood flowing in the filaments and to convey the food particles to the mouth. Younge (1960) has elaborately dealt with the mechanism of the food movements to the palps from the water current generated by the ciliary movement of the ctenidia.

4.4.1.6 Excretory System

The excretory system consists of the paired nephridia and numerous small pericardial glands (Plate 4.2B) projecting from the walls of the auricles. The nephridia consist of two large symmetrical pouch-like sacs occupying either side of the hinder half of the visceropetal mass. Each opens into the pericardium by a wide duct and to the exterior by a minute pore. They intercommunicate by a wide channel beneath the auricles. In outlines each is roughly triangular, the apex passing into the channel under the auricle, while the elongated base looks towards and forwards coinciding with the base of the anterior third of the gill of that side, and thus conforming to the inclination of the gill.
The outer wall of the nephrnidium (Plate 4.2B) is thin and membranous; it is fused with the body wall, as is also the most anterior portion of the inner wall, namely, that strip extending from the base of the gill to the viscero-pedal mass. From this line it runs back, overlying and in contact with the hinder part of the gonad, gradually narrowing as it approaches the auricle. The renal aperture is a minute oval slit like opening with sphincter muscle. It opens immediately below the genital aperture within an inconspicuous lipped slit placed at the junction of the inner plate of the inner gill with the visceral mass at a point about midway between the ventral border of the latter and the base of the foot.

Each nephridium consists of a glandular and non-glandular portion. By separating the right and left ctenidia and reflecting each, the glandular region is seen as narrow, elongated coloured strip, yellow or pale brown or even dark dull red, bordering the anterior part of the inner base of each gill. It consists of spongy tissue, occupying the anterior angle formed by the meeting of the inner and outer walls of the organ, and the secretion passes from the cavernous chambers of the glandular region directing into the spacious cavity of the main or non-glandular portion. The passage connecting the right and left nephridia lies beneath the auricles. It is a wide tunnel with thin membranous walls, bounded behind by the lower part of the pericardium. While in front its wall lies against the visceral mass below fusing with the body wall and forming part of the root of the adductor embayment of the suprabranchial chamber. The renopericardinal tubules are a pair of wide lateral prolongations of the precardiac part of the pericardium, thin walled and membranous and directed forwards. Each gradually narrows towards the anterior end where it opens into the non-glandular part of nephridium.

The aperture is a curved slit, with the concavity facing towards the ventral aspect. It has got one lip, the tube opening at a very acute angle. It is situated upon the inner wall of the nephridia. A small area around is tinted with brown pigment. The presence of accessory pericardial glands on the walls of auricles is said to have excretory function. These glands are dark brown in colour. The lower or auricular end of the pericardium is also glandular. Its epithelium is thrown into folds formed of granular vacuolated
cells of the same character as those of the nephridium.

4.4.1.7 Circulatory System

This consists of a heart with a series of arteries, which lies above the adductor, being contained in a pericardium (Plate 4.3A) and consisting of a single ventricle, (Plate 4.3A) a pair of contractile thin walled auricles (Plate 4.3A), one on each side. Blood circulation is by contraction into the anterior and posterior aorta (Plate 4.3 A). The latter is short and serves the adductor, rectum and the anus, the rest of the body is supplied by the anterior aorta, which gives off a series of minor arteries (Plate 4.3A). These open into the sinuses or blood spaces in which blood slowly circulates. The aorta finally communicates with a pair of large blood vessels that run around the margin of each mantle lobe. Deoxygenated blood is collected in veins (Plate 4.3A), which carry it either into the gills or excretory organs. Blood flows round the organs and is purified by removal of waste products of metabolism. From the nephridium (Plate 4.3A) a pair of accessory hearts pumps it into the marginal vessel of the mantle. Finally blood, from the mantle together with that from the gills returns to the heart through efferent branchial vein (Plate 4.3A) by way of auricles, the blood is colourless.

4.4.1.8 Reproductive System

The sexes are separate except in occasional cases. The gonads are paired but asymmetrical they form a thick envelop covering the stomach, liver and the first two sections of the intestine, connecting a greater part of the outside of the proximal portion of the viscero-pedal mass (Plate 4.2). The gonads do not hide the byssal gland. When the viscero-pedal mass is viewed from the right side of the byssal gland it is seen as a broad band reaching from the base of the foot back-wards to the right retractor muscle. This band appears to divide the left gonad into a larger part dorsally and a ventral smaller portion. No portion of the reproductive glands extends into the foot proper or into the mantle.

The male and female gonads are practically indistinguishable. Both are creamy yellow in colour. The male gonad in some cases is rather
paler than the female. The gonads, testes or ovaries as the case may be, consist of branched tubuli with myriads of succate caeca, the alveoli. The spermatozoa and ova develop in these. The accumulated ripe gametes fill these alveoli and tubuli and later pass into three trunks, which converge into one just within the external genital aperture (Plate 4.2B). The genital aperture is a horizontal slit located at the junction of the inner plate of the inner gill opening dorsal with renal aperture.

4.4.1.8 Nervous System

The laterally symmetrical nervous system has three pairs of ganglia (Plate 4.3B) the cerebral ganglia at the sides of the oesophagus, (2) the pedal joined to form a single ganglion at the base of the foot and (3) a pair of large visceral or parieto-splanchnic ganglia lying upon the anterior surface of the adductor. The stout paired cerebrovisceral connectives (Plate 4.3B) link the cerebral ganglia with the parieto-splanchnic ganglia, while a pair of cerebro-pedal connectives (Plate 4.3B) joins the cerebral ganglia with the pedal nerve mass. The cerebral ganglia are supra-oesophageal in position, and a nerve cord or commissure passing over the oesophagus connects the two cerebral ganglia (Plate 4.3B). A single stout transverse visceral commissure forms the two parieto-splanchnic ganglia (visceral ganglia) (Plate 4.3B). The cerebro-visceral connectives taking their rise at the posterior end of the cerebral ganglion, each passes backwards and downwards bound within the visceral mass till it merges opposite the upper angle of the base of the foot. It passes ventrally over-laid by the renal sinus entering the tissue at the base of the gills. It turns slightly forwards still passing ventrally and ends in its respective parieto-splanchnic ganglion. The cerebral ganglion of each side gives off anteriorly a stout nerve, the anterior common pallial. This bifurcates forwards. The outer branch (external pallial nerve) runs along the pallial edge, unites and anastomosing with the corresponding external pallial branch of the posterior common pallial trunk. The cerebral ganglia innervate the lateral palps and the otocysts.

The cerebro-pedal connectives arise from the posterior and outer sides of the cerebral ganglia and run downwards within the visceral
mass just behind the levator muscles of the foot to the pedal ganglion. They lie close together in their course. Four principal nerves arise from the pedal ganglion (Plate 4.3B), which innervates the foot and the byssal gland. Each of the visceral or parieto-splanchnic ganglia receives from above the stout cerebro- visceral connective, the two ganglia being themselves united by a single transverse visceral commissure (Plate 4.3B). Each ganglion also gives off two stout distributory nerves an anterior lateral and a posterior lateral. Each branchial nerve (Plate 4.3B) leaves the ganglion at the anterior lateral corner, turns down into the base of the gills and then backwards to the posterior tips following the afferent vessels. The posterior pallial nerves (Plate 4.3B) emerge from the posterior end of the visceral ganglion; from the base of each, a stout nerve passes straight back till it reaches the pigmented pallial sense organs of its respective side, a little anterior to the anus.

The common pallial trunk passes backwards and outwards bifurcating; the external branch, the larger, is the external pallial nerve. The inner branch follows a median course but divides. The outer of the resultant nerves becomes the pallial nerve; the inner, internal pallial nerve. By the ramification of these three nerves in the muscular marginal region of the mantle and by their anastomosing, a complex network of nerves termed 'pallial plexus' is formed.

4.4.2 HISTOLOGY OF THE MANTLE

4.4.2.1 Mantle Histology

The mantle of pearl oyster consists of two identical lobes, right and left, united dorsally along the hinge line 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) (Plate 4.4).

4.4.2.1.1 Marginal Zone

The free margin of the mantle lobe was thick, pigmented and fringed with tentacles. The marginal mantle was composed of the inner (IF), middle (MF) and outer (OF) folds (Plate 4.4). The outer and middle folds were separated by the periostracal groove (PG). Morphologically the folds were
similar but functionally different. The periostracal groove has got stratified epithelial cells (SEP) and periostracal secretions (PS).

**Inner fold:** the inner fold (Plate 4.4) was larger than the other two folds of the marginal mantle. It was covered with a single layer of ciliated epithelium (CE) (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. Acidophillic secretory cells (AS) measuring 3-5 μm were less while the wandering cells (WC) were more in the sub-epithelial cells.

**Middle fold:** The inner margin of middle fold (Plate 4.9A) was constituted like the inner margin of inner fold, but in the latter the epithelium was ciliated (20 μm) and columnar (CC) (Plate 4.9) in nature with pigmentation. Wandering acidophillic mucous cells (BS) were comparatively more at the tip of the middle fold (Plate 4.6B). Granulated acidophillic cells (AS) were also present. The ciliated columnar epithelium (CE) (25 μm) of the inner margin of the fold further elongated near the periostracal groove (PG) (Plate 4.5B) and reached the size of 35 μm, while at other places, they were cuboidal, brush-bordered (7-15 μm) and non-ciliated.

**Outer fold:** The outer surface of the fold was covered with specialized cells (NE). Elongated (30-35 μm) stratified columnar epithelial cells (SEP) 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) (NE) (Plate 4.5A) containing basophillic cytoplasm was present on major part of the outer fold, becoming elongated (10-20 μm) towards the tip. Basophillic cells (BS) (Plate 4.5B) occurred more on the sub-epithelial layer near the periostracal groove. Mucous cells (MU) (Plate 4.5B) were more on the inner margin of the fold, and the acidophillic cells (AS) (Plate 4.5A) towards the tip.

**Mantle isthmus:** Mantle isthmus (Plate 4.9B) or dorsal mantle consisted of non-ciliated columnar epithelium (NCC) (30-45 μm) with muscle fibres scattered below. On the dorsal side a few secretory cells (SC) were
present, and can be observed when stained in Ehrlich's haemotoxylin eosin. Sub-epithelial secretory cells were totally absent.

4.4.2.1.2 Pallial Zone

The outer epithelial cells were low columnar, nonciliated (LC) (Plate 4.8A) and small (4 - 8 µm) than the ciliated inner epithelial cells (CE) (10- 30 µm) (Plate 4.8A). In between was the muscular connective tissue (MC) (Plate 4.8A). In the outer epithelial cells of the pallial mantle, sub-epithelial layers with secretory cells (SC) and large vacuolized / porous secretory cells (VC) were present. The sub-epithelial secretory cells (SC) (Plate 4.8B) were present in the inner epithelium of the pallial mantle. The acidophilic mucous cells and granulated acidophilic secretory cells (AS) were encountered both in the outer and inner epithelial cells of the pallial mantle (Plate 4.8A & B).

4.4.2.1.3 Central Zone

The outer (shell) side of the central mantle was lined with low columnar epithelium (CE) (10- 15 µm) (Plate 4.7A). The epithelial layer contained acidophilic secretory cells (AS) and basophilic mucous cells (MU) (Plate 4.7 A). The inner margin of the central mantle is non pigmented having low columnar epithelium (CC) cells (Plate 4.7 B). Subepithelial secretory cells (SC) (Plate 4.7 B) are also seen in the inner margin of the central mantle region. Histologically, the secretory cells (SC) of inner epithelium of the central mantle (Plate 4.7B) looked similar to those of the inner epithelial cells (Plate 4.7B) of the pallial mantle.

4.4.3 PEARL-SAC FORMATION AND ITS HISTOLOGY

Formation of pearl-sac was observed in the wax nucleus implanted in the gonad of the 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 (Table 4.1).

The histological studies of the implanted graft on day 2 (Plate 4.13 A) showed that the inner epithelial cells (IEP) was not fully disintegrated

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while the outer epithelial cells (OEP) facing the wax nucleus was observed to be slightly proliferating.

The graft tissue on day 4 showed proliferation stage (Plate 4.13B) (roundish, acidophilic and larger sickle and spindle shaped) basophilic secretory cells (AS, BS) in the subepithelium. These secretory cells were seen to be coming out of the broken walls of the vacuolated porous cells (VC) of the outer epithelium towards the wax nucleus (WN).

A thin film of nacreous coating was found deposited on the nucleus within 18 days on a 4 mm wax nucleus (Plate 4.14). Histological studies of the nacreous pearl-sac epithelium (PE) in the male gonad (GN) of the pearl oyster showed acidophilic cells (AS) in low magnification. Under higher magnification (Plate 4.14B) the hexagonal crystalline secretion (CR) on the wax nucleus, and acidophilic secretory cells (AS) in the pearl sac epithelium (EP) covering the wax nucleus were observed.

In further higher magnification, this nacreous pearl sac (Plate 4.15 A) with pearl coating on the wax nucleus (WN) was observed that the concentration of granular acidophilic secretory cells (AS) in the sub epithelium of the nacreous pearl sac. The proliferated cells (haemocytes) (HC) have replaced part of the nacreous pearl sac epithelium and pearl coating (PWN) showing that the pearl sac has become a part of the gonad of the host oyster.

Histological studies of the nacreous pearl sac (Plate 4.15B) formed on the shell bead nucleus which had produced good and lustrous pearl showed, the presence of more cuboidal, flattened non-ciliated epithelial cells (CP) along with large secretory cells (4-6 μm) (Plate 4.15B). The cells were similar to the cells of the muscular tissues of the gonad (GN). 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 (Plate 4.16A), which were produced with the wax nuclei in the laboratory, tall, ciliated columnar epithelial cells (CCP) (30-35 \( \mu m \)) were well distributed. Congregations of cells resembling haemocytes (HCC) were also present in some areas of the epithelium (Plate 4.16A). Basophilic mucous cells (BS) with granular inclusions were common. Acidophilic cells (AS) with large secretory granules were present in some parts of the periostracal pearl-sac.

The pearl oyster implanted with 4 mm shell bead nucleus and 2 mm x 2 mm graft has produced good lustrous pearls (Plate 4.16B) after 4 months whereas the abnormal pearl sac produced “D” quality pearls.
4.5 DISCUSSION

The anatomy of Pinctada fucata is comparable to that of \textit{P. vulgaris} by Purchon (discussed in Kuwatani, 1965 a) who compared the anatomy of \textit{P. vulgaris} and \textit{P. martensii} and of Herdman (1904). Herdman (1904) described the anal pappila of \textit{Margaritifera vulgans} as five fold. Shiino (1952) had drawn the structure of the anal papillae of \textit{P. martensii} (Dunker). In \textit{P. fucata} (Gould) the anal papillae is trifold. In \textit{M. vulgaris} there are three nerves enervating from the pedal ganglion wherein \textit{P. fucata} (Gould) has 4 nerves. Herdman (1904), Shiino (1952) noticed the renogenital aperture in \textit{M. vulgaris} and \textit{P. martensii}. In \textit{P. fucata} (Gould) the aperture is very difficult to trace. Kuwatani (1965 a) differentiated the stomach of \textit{P. martensii} from \textit{P. vulgaris}. According to him, in \textit{P. vulgaris} the left intestinal groove arise from the pouch leading to the origin of the groove. In \textit{P. martensii}, the groove however is at the point of coming to the tongue from the major typhlosole in the second embayment. Chellam (1983) stated that the stomach content of \textit{P. fucata} contained straight hinge stage bivalve larvae from 27.5 to 115 \textmu m in dorso ventral axis and 37.5 to 125 \textmu m in antero–posterior axis. The larvae with umbo ranged in size from 162.5 to 232.5 \textmu m in dorso ventral axis and 200 – 275 \textmu m in antero–posterior axis. In \textit{P. martensii} the charcoal particles taken into the oesophagus were 30 \textmu m and 17.5 \textmu m respectively. It is inferred from this study that the stomach width as well as the oesophagus is larger in \textit{P. fucata}.

The regional as well as functional differentiation of the mantle is marked in \textit{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 \textit{Pinctada fucata}. A similar type of stratified columnar cell has been recorded in \textit{P. maxima} (Dix, 1973) and in \textit{P. margaritifera} (Zahab et al., 1992). 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 probably a sensory function for this fold.

The outer epithelial cells of the pallial and the central mantle were small and non-nucleated with large vacuolized/porous secretory cells. Basophilic mucous cells and granulated acidophilic secretory cells were found scattered in the inner and outer 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. This was same in P. maxima (Dix, 1972; However, Zahab et al. (1992) found that the cells of the outer fold of the pallial epithelium was mucous secretory in nature in P. margaritifera.

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 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. According to a recent study by Compos et al. (2000) who studied the ultrastructure of the pearl sac epithelium of P. margaritifera, the abnormal pearl sac was due to mineralisation disturbances related with the production of laminated organic structures in nacreous layers of the shell and the pearl. They further put forward a hypothesis that the epithelium which supplies the crystal deposit may also consists of areas of cells involved in periostracum production. This cell could be originally present in the mantle tissue used as graft or differentiated during the formation of pearl sac epithelium owing to some specific factors. They were of the opinion that the abnormal secretion
was a response to either a wound healing mechanism or by the introduction of a foreign body during the grafting.

From the histological studies of the implanted graft tissue on day 2 in the gonad of the oysters the inner epithelial cells were not fully disintegrated while the outer epithelial cells facing the wax nucleus almost is in proliferating stage for the formation of pearl sac over the wax nucleus. The most significant observation was the histology of the 4th day graft on the wax nucleus in P. fucata gonad, which showed formation of pearl sac from day 4 onwards. This is in agreement with the works of Awaji and Suzuki (1995) and Wada (1996) for Pinctada fucata martensii and Garcia-Gasca et al. (1994) for Pinctada mazatlanica. The sickle and spindle shaped larger basophilic muscle cells and roundish acidophilic cells formed from the graft was similar to those type of cells produced by in vitro tissue culture of outer mantle epithelium of P. fucata by Machii (1974)

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 indicates their dual function, in the secretion of conchiolin. Ojima (1952) is of the opinion that the middle fold of mantle is the main portion for secretion of conchiolin. 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 the conchiolin and calcium (Ojima 1952).
A. *Pinctada fucata* (Gould): Figure illustrating the morphology and anatomy of the oyster

B. Figure illustrating the right shell remove showing morphology and anatomy of the oyster

A. *Pinctada fucata* (Gould): Digestive system


B. Respiratory, excretory and reproductive systems

A. *Pinctada fucata* (Gould): Circulatory system


B. Nervous system

A. *Pinctada fucata* (Gould): Digrammatic view of sites and different regions of mantle selected for histological studies

Central mantle (CM); Pallial Zone (PZ); Marginal Zone (MZ); Central Zone (CZ); Adductor muscle (AM); Pallial mantle (PM); Marginal mantle (MM); Inner fold (IF); Middle fold (MF); Outer fold (OF); Periostracal secretion (PS) and Mantle isthmus (IS).
A. *Pinctada fucata* (Gould): Histological section of the three folds of the mantle, inner (IF) middle (MF) and outer (OF) folds of the marginal mantle, periostracal groove (PG), stratified epithelial cells (SEP), and periostracal secretion (PS).

Wandering cells (WC), strong band of muscles (MS), and pigmented epithelium (PE), of the inner fold of the marginal mantle, periostracal groove (PG), stratified epithelial cells (SEP), and periostracal secretion (PS).

B. Histological section of the middle and outer folds of the marginal mantle

Pigmented epithelium (PE), Wandering cells (WC), of middle fold and non pigmented epithelium (NE), basophilic cells (BS), and (MU) mucous cells.
A. *Pinctada fucata* (Gould): Histological section of the inner marginal mantle fold

Wandering cells (WC), strong band of muscles (MS), and pigmented epithelium of the inner fold of the marginal mantle (PE). Acidophilic cells (AS), strong band of basophilic cells (BS), and (CE) ciliated epithelium.

B. Histological section of middle mantle fold

Inner marginal mantle fold

Basophilic cells (BS) found in the tip of the middle mantle fold
A. *Pinctada fucata* (Gould) : Histological section of the outer margin of the central mantle

Concentration of mucous cells (ML) and acidophilic granular secretory cells (AS) below the columnar epithelium (CE).

B. Histological section of the inner margin of the central mantle

Inner non-pigmented low columnar epithelium (CC) and sub-epithelium secretory cells (SC).
A. *Pinctada fucata* (Gould) : Histological section of the pallial mantle

Outer non-ciliated low columnar epithelium (LC), sub-epithelial secretory cells (SC) vacuolated porous secretory cells (VC), muscular connective (MC) and (AS) acidophilic secretory cells.

B. Histological section of the pallial mantle

Inner ciliated pigmented low columnar epithelium (CE), sub-epithelial secretory cells (SC) and (AS) acidophilic secretory cells.
A. *Pinctada fucata* (Gould): Histological section of the middle and outer folds of the marginal mantle

Wandering secretory cells (WC) of the middle mantle fold, non-pigmented epithelium (NE) of the outer fold and collated non-pigmented columnar epithelium (CC) and sub-epithelial mucous cells (BSS).

![Histological section of the middle and outer folds of the marginal mantle](image)

B. Histological section of the mantle isthmus.

Strongly basophilic non-ciliated columnar epithelium (NCC) and secretory cells (SC).

![Histological section of the mantle isthmus](image)
A. *Pinctada fucata* (Gould) : Setup for pearl oyster surgery

B. Narcotization of pearl oyster surgery for pearl sac formation study
A. *Pinctada fucata* (Gould) : Preparation of mantle graft tissue for pearl sac formation study

B. Implantation of graft tissue in to the gonad of the pearl oyster
A. *Pinctada fucata* (Gould): Insertion of wax nucleus into the gonad of the pearl oyster for pearl sac formation study

B. Implanted oysters kept in controlled running water system for post-operative care and pearl sac formation study
A. *Pinctada fucata* (Gould): Studies on pearl sac formation using wax nucleus

Second day implanted graft tissue: Spindle shaped secretory cells (SC) acidophilic and basophilic cells (BS) in the sub-epithelium and broken cells of vacuolated cells (OEP), inner epithelial cells (IEP).

B. Fourth day implanted graft tissue: Spindle shaped secretory cells (SC) acidophilic and basophilic cells (BS) in the sub-epithelium and broken cells of vacuolated cells (VC) in the outer epithelium through which secretory cells come out towards wax nucleus (WN).
A. *Pinctada fucata* (Gould) : Studies on pearl sac formation using wax nucleus

Pearl coating (PWN) formed on 18th day on 4mm wax nucleus (WN), pearl sac epithelium (PE) in the male gonad of pearl oyster.

B. Structure of pearl secretion on wax nucleus

Crystalline secretion (CR) on the wax nucleus, acidophilic secretory cells (AS) in the pearl sac epithelium (EP) covering the wax nucleus.
**A. Pinctada fucata** (Gould): Histological section of the normal pearl sac

Concentration of granular acidophilic secretory cells (AS) in the sub-epithelium of nacreous pearl sac formed on wax nucleus (WN), proliferated cells (haemocytes (HC) which have replaced part of the nacreous pearl sac epithelium and pearl coating (PWN).

**B. Histological section of the normal pearl sac formed on shell bead nucleus**

Cuboidal flattened epithelium (CP) of the normal pearl-sac formed on the shell bead nucleus implanted along with mantle graft (resulted in an "A" quality pearl). Haemocytes (HC) of the gonad tissue extended into the pearl-sac epithelium, acidophilic secretory cells (AS) and (B) basophilic secretory cells.
A. *Pinctada fucata* (Gould): Histological section of the abnormal pearl sac

Tall columnar ciliated epithelium (CCP) of a periostracal pearl sac formed on wax nucleus, cells resembling haemocytes (HCC), basophilic mucous cells (BS), acidophilic secretory cells (AS); with large secretory granules.

B. Normal nacreous pearl formed by normal pearl sac on shell bead nucleus

Implanted oyster with quality pearl (P) coating on nacre in a spent oyster
Table 4.1 *Pinctada fucata* (Gould): Duration of pearl sac formation

<table>
<thead>
<tr>
<th>Size of wax nuclei</th>
<th>Duration of pearl sac formation</th>
<th>Day of pearl coating observed on wax nuclei</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.12 ± 0.11</td>
<td>3 - 7 days</td>
<td>Not observed</td>
</tr>
<tr>
<td>4.09 ± 0.10</td>
<td>4 - 10 days</td>
<td>Day 18</td>
</tr>
<tr>
<td>5.09 ± 0.06</td>
<td>6 - 12 days</td>
<td>Not observed</td>
</tr>
</tbody>
</table>