Mar. Res. Indonesia Vol.34, No.1, 2009: 17-26

OOCYTES OF *HOLOTHURIA LEUCOSPILOTA* (ECHINODERMATA: HOLOTHUROIDEA): AN ULTRA STRUCTURAL STUDY

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Received: 14 August 2008 Revised and Accepted: 10 April 2009

ABSTRACT

Ovaries of *Holothuria leucospilota* consist of simple tubules, within which oocytes grew and reached maturity. The oocytes development started at the tubule lining and as the development proceeded, the oocytes increased in size and moved to the tubule lumen. An ultra-section-method was applied to describe ultrastructural changes in the oocytes during their development, leading to the knowledge on gametogenesis of sea cucumber *H.leucospiota*. Primary previtellogenic oocytes embedded in tubule lining was the earliest stage, which were characterized by the presence of nuage. Prior to yolk formation, clusters of mitochondria appeared in the ooplasm. This may indicate the need for energy to produce yolk substance. When yolk bodies were common in vitellogenic oocytes, nuage became invisible and mitochondria were no longer in groups. As development progressed, abundant microvilli appeared on the surface of vitellogenic oocytes, but less common on postvitellogenic oocytes when maximum growth had been attained. This structure was suggested to facilitate size increase and functional efficiency of oocyte surface.

INTRODUCTION

Holothurians develop their gametes within gonad tubules. Reports on anatomical structures and development of gonads of *H.leucospilota* have been provided in detail in several publications including Purwati and Luong-van (2003), Purwati (2001) and Franklin (1980). During development, particular organelles within an oocyte appear consecutively, which denotes changes in fine structures. Nuage is a distinctive organelle in previtellogenic oocytes whereas yolk is characteristic of vitellogenic and postvitellogenic oocytes. In postvitellogenic oocytes, cortical granules appear in the periphery of oocytes (Ohshima, 1925; Franklin, 1980; Smiley and Cloney, 1985; Kessel, 1987; Smiley, 1988; Eckelbarger and Young, 1992; Tyler *et al.*, 1994).

Follicle cells in holothurians bordered adjacent oocytes. These cells may derive from the inner epithelium of ovary and are usually ciliated as appear in *Stichopus californicus* (Smiley, 1988), *Bathyploten natans* (Tyler *et al.*, 1994) and the 10 species investigated by Eckelbarger and Young (1992). Connection between oocytes and follicle cells is maintained through a protuberance (Maruyama, 1980; Smiley, 1988; Smiley, 1990).

General shape of follicle cells and whether these cells migrate during oocyte development may differ amongst species. In deepsea aspidochirote *B.natans* (Tyler *et al.*, 1994), *Hasenthuria benti* and *Holothuria occidentalis* (Eckelbarger and Young, 1997), follicle cells possess podocytes, but not in other species observed by Eckelbarger and Young (1992), nor in *H.leucospilota* or S.*chloronotus* (Franklin, 1980) or *S.californicus* (Smiley, 1988). In the last species, follicle cells begin to encircle oocytes individually in size of approximately 40 µm, and remain in the tubule lining (Smiley, 1988; Smiley and Cloney, 1985). In *H.leucospilota* populating Heron Island (Franklin, 1980), follicle cells have been reported to differentiate early, occupying space among oogonia which are embedded in connective tissue of the tubule lining. Follicle cells remain at the tubule lining when the oocytes move to the tubule lumen. However, in other population of *H.leucospilota* (Maruyama, 1980), *S.japonicus* (Drosdov *et al.*,

DOI: 10.14203/mri.v34i1.519

1991) and *Psolus fabricii* (Hamel *et al.*, 1993), follicle cells are reported to maintain encircling the oocytes until spawning.

Research on holothurian reproductive biology intensified during the 1980s with increase focus on oocyte ultra structures. This research area benefits experiments where criteria of mature gametes are required. Such investigation is also advantageous as it allows comparative studies, both inter- and intra-species, determining whether species and habitat variations influence fine structure of gonads and gametes, which then may lead to a clearer understanding of how the animals evolve. This paper investigates the detailed characteristics of oocytes of *H.leucospilota* collected from tropical water of East Point, Darwin, Northern Territory, Australia. Observations are focused on structural changes during development.

MATERIALS AND METHODS

H.leucospilota studied were those inhabiting intertidal zone of East Point, Darwin (12° 26' S, 130° 51' E). Samples of gonads prepared for ultrastructural studies were those collected from the same gonads as for histological studies (See Purwati 2001; Purwati and Luong-van 2003). Fresh fractions of ovarian tubules from different development, approximately 5 mm long, were cut and fixed in 3% glutaraldehyde in filtered seawater for an hour at room temperature. Fixed samples were then rinsed 3 times with filtered seawater to remove fixative. Subsequently, the samples were stored in filtered seawater at 4° to 6° C until the next processing. Post-fixation was applied in 1% osmium tetroxide (OsO4 in filtered seawaters) at room temperature for an hour, rinsed with tap water three times and separately dehydrated for Transmission Electron Microscope (TEM).

Dehydration used Reicher Lynx tissue processor in series of ethanol followed by acetone and acetone-resin mixture. Samples were then embedded in Spurr's Resin and cured at 60° C for 72 hours. Ultra thin sections were prepared in LKB Bromma, Nova ultra microtome and stained in uranyl acetate and lead citrate. Examination was made using Joel JEM 1200 EX TEM.

RESULTS

Previous histological studies taken on the same population of *H.leucopsilota* (Purwati 2001, Purwati & Lung-van 2003) drew conclusion that oocytes move from the periphery to the lumen of gonadal tubules during development. Simultaneously, oocytes increase in dimension and complexity (Fig. 1). To characterize stages of development, vitellogenesis (or yolk synthesis) was used as a parameter which has been adopted in most previous studies including Smiley and Cloney (1985), Eckelbarger and Young (1992) and Tyler *et al.* (1994). Previtellogenic (PO), vitellogenic (VO) and postvitellogenic (PoO) referred respectively to stages of oocytes prior to yolk formation, during yolk synthesis and when the synthesis ceases.

POs were mostly observed in association with tubule lining, and VOs as well as postvitellogenic PoOs were located in the tubule lumen. Nuclei remained intact in all stages of oocyte development, while organelles and oocyte membrane altered. Non-ciliated follicle cells encircled VO individually, and remained so until the oocytes reached maximum growth. Figure 2 showed changing in fine structure of oocyte during development.

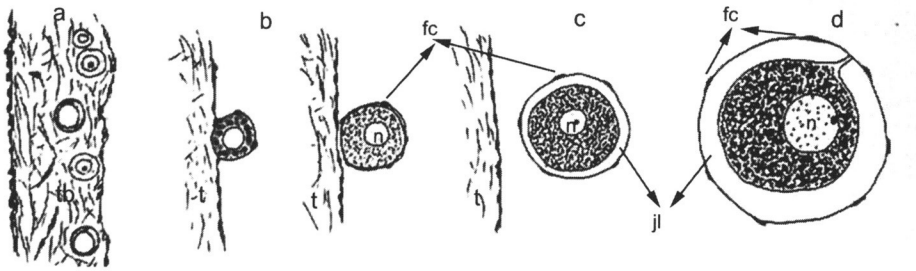

Figure 1. Diagram of oocyte movement during development (no scale). a: shows oogonia embedded in the tubule lining; b: primary previtellogenic oocyte, ready to detach from the tubule lining; c: primary vitellogenic oocyte in the periphery of tubule lumen; d: fullgrown primary oocyte in the lumen. fc: follicle cells; jl: jelly layer, t: tubule lining; n : germ cell (nucleus).

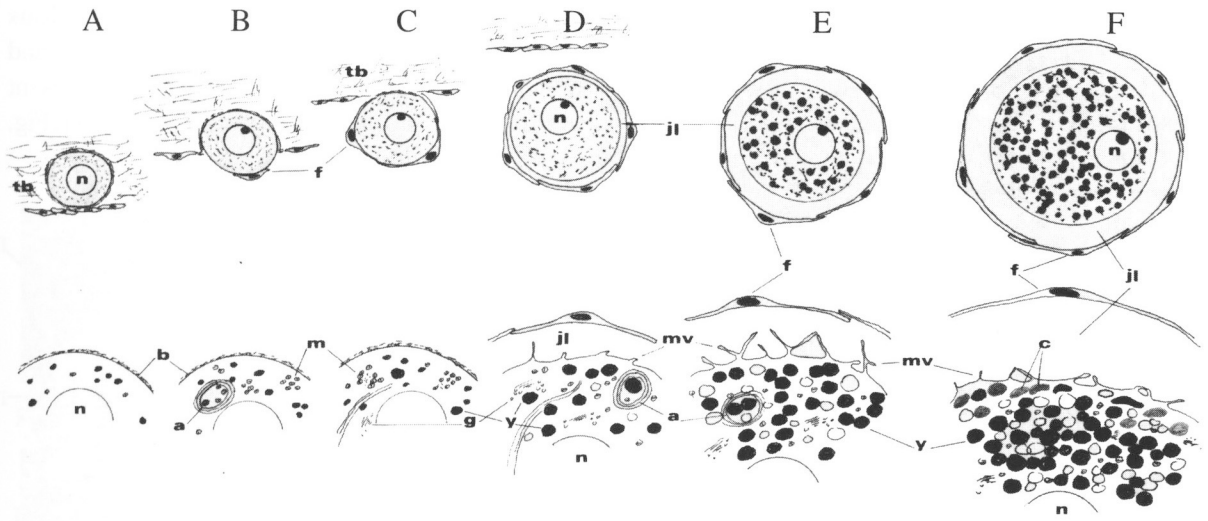

Figure 2. Diagram of oocyte development. Row below is detail of above row (no scale). A-C: previtelogenic oocyte; D-E: vitelogenic oocyte; F: fullgrown postvitellogenic oocyte. a: annulate lamella; b: oolemma; c: corticle granule; f: follicle cells; g: golgy complex; jl: jelly layer; m: mitochondria; mv: microvillie; n: nucleus; nc: nucleolus; nu: nuage; tb: ovarian tubule lining; y: yolk

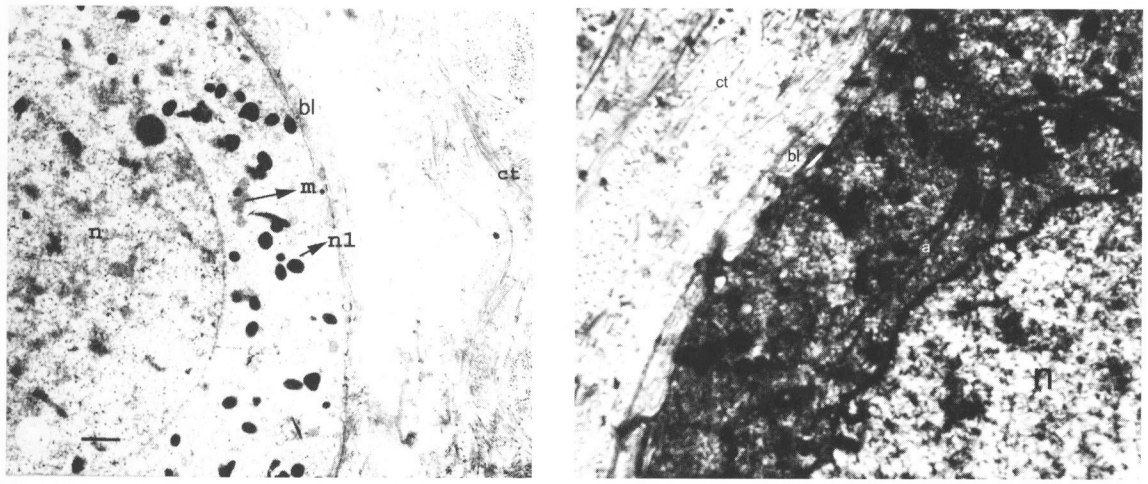

Figure 3. Previtellogenic oocyte (possibly oogonia) embedded in tubule lining (scale bar: 1,4 µm). a: annulate lamella; bl: basal lamina; ct: connective tissue of tubule lining; m: mitochondria; n1: nuage type 1; n: nucleus.

POs were 7 to 20 µm in diameter and commonly observed within ovarian tubules during early development stage. General surface was smooth and lacked of microvilli. These POs were isolated individually from the surrounding connective tissue by oolemma or basal lamina which consisted of dense fine fibers of connective tissue (Fig. 3). In oocytes attaching tubule lining, the oolemma was only observed in the area affixed to the tubule lining. Nuclei were large (10-11 µm) and contained chromatin distributed throughout the nucleoplasm. Single spherical nucleolus, approximately 2.5- 3.0 µm in diameter, was situated on the periphery or in association with the inner nucleo-membrane (Fig. 3).

Multinucleoli as reported in *T.briareous* (Ohshima, 1925) were not observed. Nuage granules were obvious in the cytoplasm, either yolk bodies or vacuoles were absent. POs containing groups of mitochondria were frequently observed. Jelly layer was absent. Follicle cells were only present at the apical part of oocytes facing the lumen.

When POs reached 20-30 µm and prior to their liberation from the tubule lining and yolk synthesis, follicle cells encircled oocytes individually.

Occasionally, follicle cells were observed overlapped. Space gradually developed between PO membrane and follicle layer, followed by the rise of microvilli on the oocyte surface.

VOs in growing tubules and PoOs dominating fecund tubules were distinguished by the striking appearance of the yolk granules, while nuage were inconspicuous. Diameter of VOs varied greatly, up to 90 μm to 100 μm which was considered to be fullgrown PoOs. Microvilli were common. Jelly layer (occupying space between membrane and follicle cells) and follicle cells enclosed each oocyte. In late VOs and PoOs, another type of granules namely cortical granules, were observed mainly in the periphery region of ooplasm.

Bridging structure between oocyte and follicle cells, protuberance, extended beyond oocyte and passed through the jelly layer. Protuberance contained abundant free ribosomes and microtubules. Other cellular organelles were limited (Fig. 4).

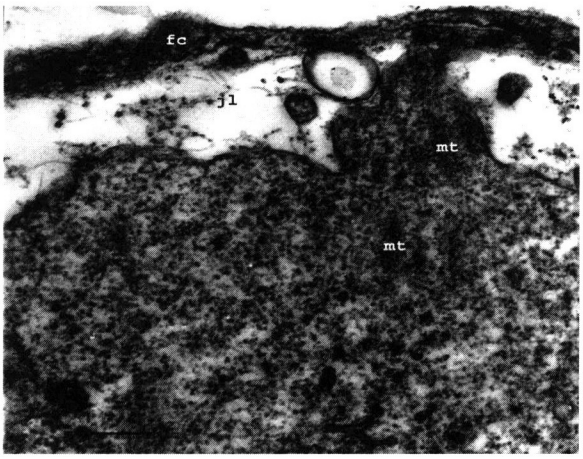

Figure 4. Area of protuberance bridging between follicle cells (fc) and oocytes (scale bar: 240 nm). Ribosoms appear as fine dense-granules. jl: jelly layer; mt: microtubules.

Below was description and condition of organelles which evolved during development.

OOCYTES

Nucleus

Nucleus or germinal vesicle of small PO was commonly 10-11 μm in diameter, and reached 48-50 μm in PoOs. Germinal vesicle breakdown (GVB) was never observed at any stage of oocyte development. Chromatin dispersed throughout the nucleoplasm. A spherical, dense granulous nucleolus, approximately 2.5-3 μm in diameter and reaching 7-8 μm in fullgrown PoOs, was present peripherally, and adhered to the nucleolemma (Fig. 3, 5).

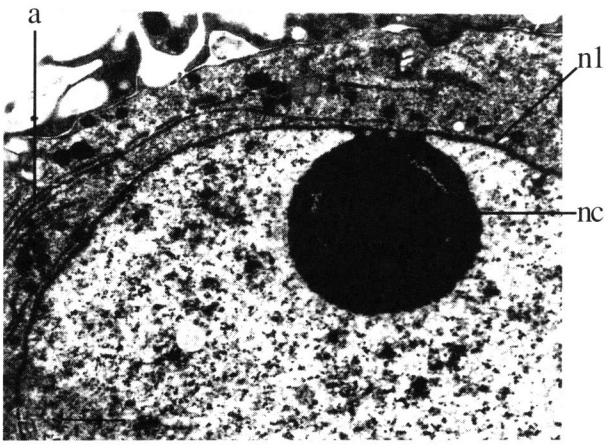

Figure 5. Previtellogenic oocyte (scale bar: 1 μm). a: annulate lamellae; n: nucleus; nc: nucleolus; n1: nuage type 1.

Annulate lamellae

Lamellar structures which performed multi-layered rings or layers were distributed throughout the cytoplasm of POs and early VO. Thickness of each layer was less than 0.01 nm. Occasionally, the lamellar rings surrounded the nucleus (Fig 5, 6).

Nuage

Nuage (Fig. 2, 3, 7) were distinctive organelles in POs. Two types of nuage, similar to those in *Eupenctacta quinqusemita* (Eckelbarger and Young, 1992), could be observed. Nuage type 1 was conspicuous with its non-membrane bound spherical bodies, fine granular outline, electron dense, approximately 250 to 450 nm in diameter. Nuage was relatively abundant and persisted until vitellogenesis. Nuage type 2 (Fig. 7) had an irregular outline, contained loose electron dense fine granules. They were more noticeable amongst groups of mitochondria. When mitochondria were distributed throughout the cytoplasm and yolk bodies appeared, nuage were hardly found.

Mitochondria

Mitochondria were generally ovoid, 250-375 nm, frequently contained electron dense inclusions. They were scattered and fewer in small POs (probably oogonia) (Fig. 7, 8).

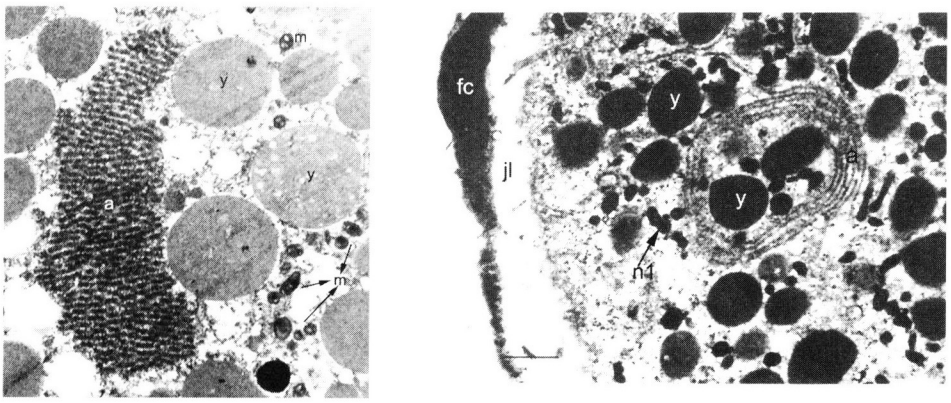

Figure 6. Different appearance of annulate lamellae (a) in vitellogenic oocytes. fc: follicle cell; jl: jelly layer; m: mitochondria; y: yolk bodies

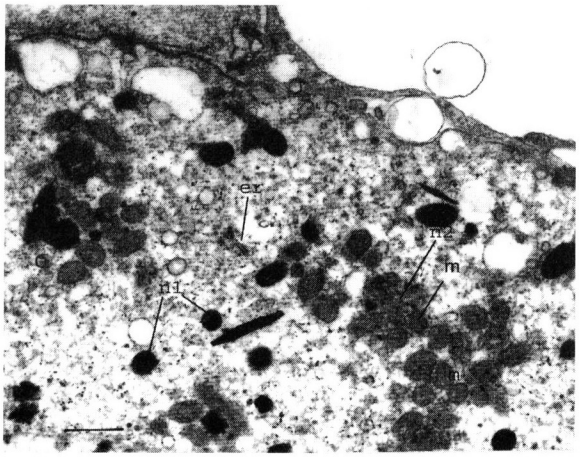

Figure 7. Previtellogenic oocyte (scale bar: 750 nm). er: fraction of endoplasmic reticulum; m: group of mitochondria; n1: nuage type 1; n2: nuage type 2.

Mitochondria multiplied intensively when POs prior to liberate from the tubule lining. Several distinctive constricted mitochondria were observed in groups throughout the cytoplasm while other organelles were scarce. There was no indication of either ring formation in which mitochondria encircled nuage type 2 as reported in *S.californicus* (Smiley, 1988), or formation layers of mitochondria alternated endoplasmic reticulum as reported in *T.briareus* (Kessel, 1966). When yolk appeared, clusters of mitochondria broke up and solitary mitochondria were scattered throughout cytoplasm.

Endoplasmic reticulum and free ribosomes

Endoplasmic reticulum were more frequently observed in short segments, and rarely found in more than three layers. The outline was rough indicating the presence of ribosomes. These organelles also frequently appeared as small vesicles in short rows distributed all through cytoplasm, mainly in VOs. Free ribosomes and microtubules were abundant in the cytoplasm and in area of protuberance (Fig. 7, 8).

Golgi complex

The Golgi apparatus were observed as lamellar structures with maximum lamellar length between 1000 and 1100 nm. They were always found with small vesicles or cisternae on the maturing side. Some vesicles contained electron dense materials. It was more likely that a greater number of vacuoles were present in an oocyte when more Golgi complex were observed. Golgi apparatus were less common in POs and became easier to find prior to the appearance of yolk granules (Fig. 8).

Yolk bodies

Yolk granules were mostly spherical or ovoid in shape and appeared as a medium granular electron dense organelle of variable size. Larger granules commonly had diameter between 1600 to 2000 nm. Yolk bodies were rarely observed within oocytes associated with the tubule lining (Fig. 6, 8).

Vacuoles

Vacuoles developed in late POs. In VOs and PoOs, this type of organelle appeared as an empty rounded space surrounded by a thin membrane, varied in size and was scattered all over the cytoplasm. Small vacuoles were likely to originate from Golgi apparatus as they were visible at the mature face of this organelle. This was observed in small oocytes (PO) that moved away from the tubule lining. As oocytes enlarged, vacuoles increased in number and size. Large vacuoles may

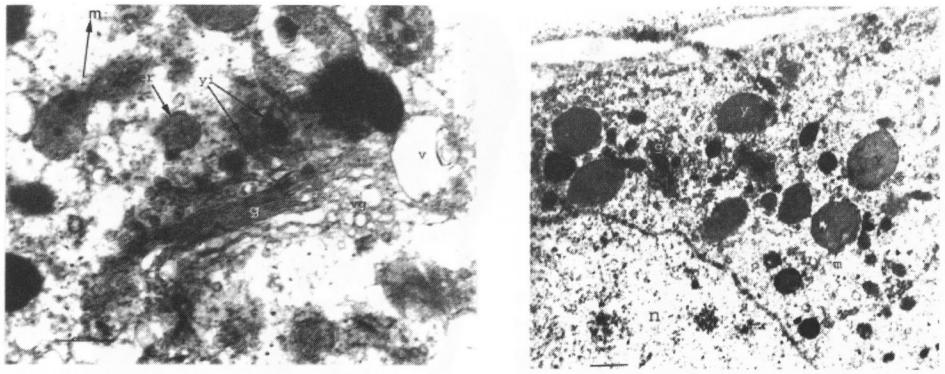

Figure 8. Golgy apparatus in previtellogenic oocytes (scale bars: 250 nm). er: fraction of endoplasmic reticulum; g: Golgy compartment; m: dividing mitochondria; n: nucleus; v: vesicle; vg: vesicle of golgy; y: yolk.

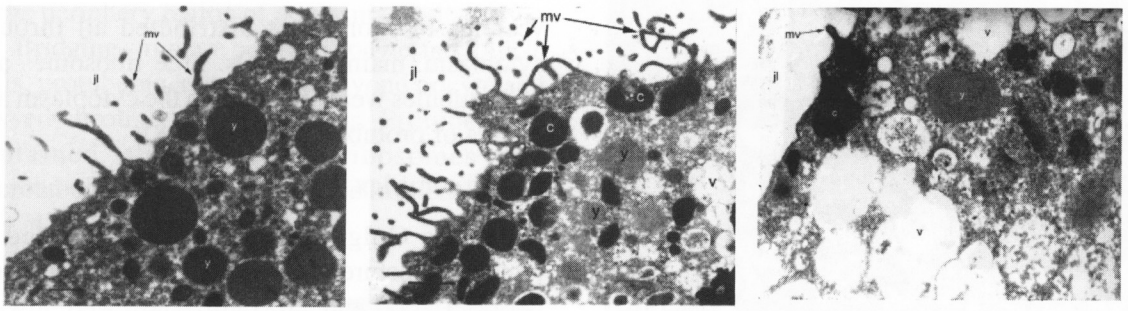

Figure 9. Oocyte surface. Left to right: previtellogenic, late vitellogenic, postvitellogenic oocytes. c: corticle granule; g: golgy complex; jl: jelly layer; mv: microvilli; v: vacuole; y: yolk.

have been the result of coalescence of small ones as two or more vacuoles were often observed undergoing fusion (Fig. 9). Some vacuoles were observed in exocytosis in VOs.

Cortical granules

Cortical granules were electron dense bodies at the periphery of fullgrown PoOs. Granules varied in shape and size, with maximum diameter reaching 0.9 μm (Fig. 9).

Microvilli

Microvilli were absent in POs but very common in VOs. These structures seemed to develop after or following the creation of space between oocyte membrane and follicle shield. Microvilli grew longer, branched and became complex in larger oocytes (VOs), but became shorter and fewer in fullgrown PoOs (Fig. 9, 10).

Jelly layer

Jelly layer was an amorphous structure that occupied the space between oocyte membrane and follicle shield. It appeared in oocytes liberating from the tubule lining and became thicker, reaching 10 μm in full-grown PoOs. Vacuole transfer from the cytoplasm (exocytosis) was frequently observed, which may have indicated jelly layer accumulation (Fig. 9, 10).

Follicle cells

When oocytes (PO and early VO) moved to the lumen, follicle cells began to isolate each of them (Fig. 10). It was apparent as if the innermost cells of tubule lining were being pushed away from the tubule lining when the nearby oocyte bulged into the lumen. These cells followed oocyte migrating to the ovarian lumen, and remained encircling until fullgrown PoOs. As oocytes increased in size, follicle cells stretched and became flatter.

Nuclei in follicle cells were relatively large but compressed. Chromatin condensed along the inner side of the nucleo-membrane. Nucleoli similar to that observed in another population of *H.leucospilota* (Franklin, 1980) were never observed. Smooth endoplasmic reticulum and mitochondria were occasionally present in the cytoplasm. Lysosomes, as in *E.quinquesemita* (Eckelbarger and Young, 1992), were frequently

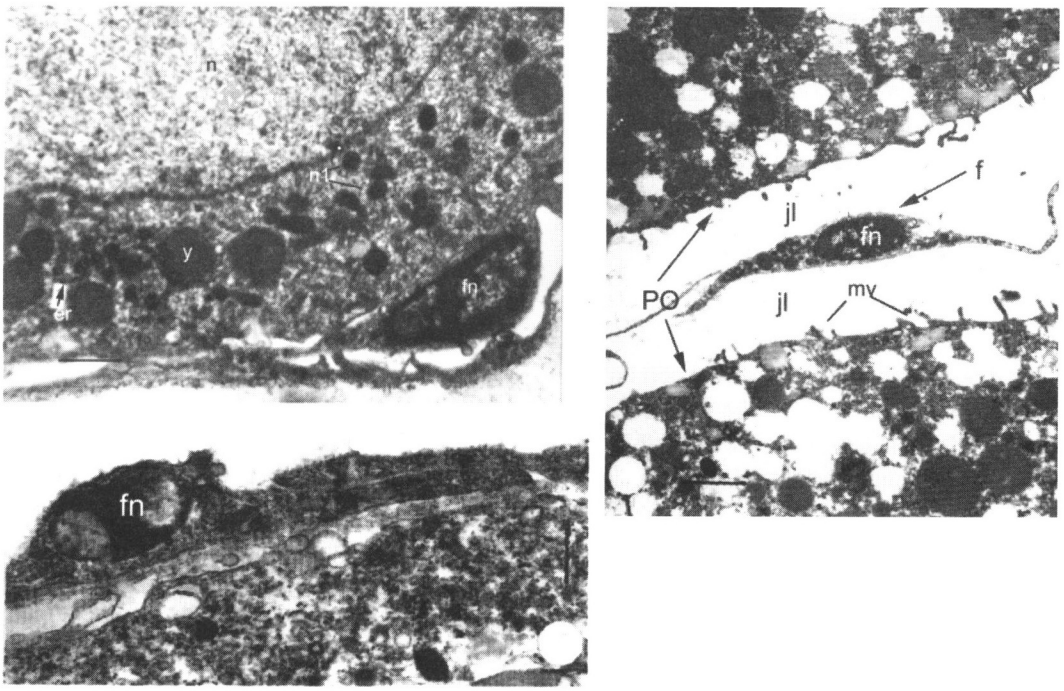

Figure 10. Follicle cell (f) in previtellogenic (upper left) and post vitellogenic oocytes (right). Scale bars: upper left: 1 µm, 200 nm (right), 400 nm. fn: nucleus of follicle cell; mc: microvilli of oocyte; n: nucleus of oocyte; n1: nuage type 1; PO: postvitellogenic oocyte; y: yolk.

visible. There was no evidence of either cillia or pinocytoses by follicle cells as has been reported for *H.leucospilota* inhabiting Heron Island (Franklin, 1980).

DISCUSSION

The largest oocytes within ovarian tubules of studied population were immature, therefore the germinal vesicles were found always intact. Previous studies have confirmed that spawned oocytes are immature fullgrown, in stage between the break down of germinal vesicle and first polar body protrusion (Drosdov *et al.*, 1991; Smiley and Cloney, 1985; Holland, 1981; Holland & Dan, 1975; Franklin, 1980; Maruyama 1980). Consequently, oocytes within tubules are prophase-primary oocytes. In spite of that meiosis has not been completed, oocytes in this stage are fertilizable. Most vertebrate oocytes, in comparison, arrest in diplotene stage of second meiotic metaphase, and remain so until the fertilization occurs (Cooper, 1985).

Oogonia, which were associated with tubule lining, were the smallest female germ cells observed in this study. Mitotic division was not observed in any oogonia, although several cells were observed in small groups. The presence of nuage distinguished them from somatic cells. Even though the origin and function of nuage remains in debate (Kessel, 1966; Franklin, 1980; Tyler *et al.*, 1994), its presence identifies young oocytes of several species in both invertebrate and vertebrates (Smiley, 1988, 1990).

In small ovarian tubules or primary tubules of *S.californicus*, oogonia are the earliest observed germ cells (Smiley, 1988). In addition to the presence of nuage, mitotic division was evident. Nuage, together with groups of mitochondria also characterize primary oocytes of *B.natans* (Tyler *et al.* 1994). These characters were similar to the present study. Meanwhile, a ring formation of mitochondria and nuage type 2 at the centre occurs in *S.californicus* (Smiley, 1988), and the formation of alternate layers of mitochondria and endoplasmic reticulae as reported in *T.briareus* (Kessel, 1966) were not observed in this study, neither in other population of *H.leucospilota* studied by Franklin (1980).

Previtellogenesis involved all changes occurring after mitotic division of oogonia until the beginning of vitellogenesis (Smiley, 1988). Clustering of mitochondria, which was thought to be typical feature in echinoderm oocytes (Smiley,

1990), indicated intensive proliferation of these organelles in accordance with the production of large amounts of energy. Multiplication of mitochondria therefore, may be used to indicate change from oogonia to primary oocytes, supporting the suggestion that energy accumulation is the first step taken by an oocyte prior to grow and develop (Anderson, 1974).

Annulate lamellae have been reported to originate in the nuclear membrane, and are present not only in gametes but also in somatic cells and even plant cells (Kessel, 1987). The function of annulate lamellae is unknown. In deep-sea holothurians species, annulate lamellae are present in all stages of oocyte development (Tyler *et al.*, 1994). The current work was not able to suggest a function, even though this organelle was common in early and vitellogenic primary oocytes. When the density of oocytes was high, this structure became invisible.

Ultrastructural observation revealed that yolk was the first material accumulated after mitochondria multiply. Yolk containing lipid-like granules or lipid droplets resembling deep holothurian species (Tyler *et al.*, 1994) and *H.leucospilota* of Heron Island (Franklin, 1980; Eckelbarger and Young, 1992), or cristalloid structures noted in *S.californicus* (Smiley and Cloney, 1985) were not observed in this study.

Small electron dense granules, coated with a single membrane similar to that of yolk bodies, were sometimes observed close to the Golgi complex. Two or three vesicles were frequently observed in fusion. The presence of these vesicles may indicate that Golgi produced at least part of the yolk bodies within the cytoplasm. This is also the case in *H.leucospilota* of Heron Island (Franklin, 1980) and more likely in *T.briareus* (Kessel, 1966) and *B.natans* (Tyler *et al.*, 1994).

Vacuoles were observed coexisting with yolk granules. At least a part of the vacuoles was apparently produced by Golgi. Apparently, vacuoles were involved in exocytosis, which may concern with jelly layer production. It has been suggested that the vacuoles contained secretory materials which may be important in accumulation of jelly materials. As in *T.briareus* (Dendrochirote: Cucumariidae) (Kessel, 1966), *S.californicus* (Smiley, 1988) and *B.natans* (Aspidochirote) (Tyler *et al.*, 1994), therefore, it is concluded that the jelly material is produced by the oocytes. On the other hand, Franklin (1980) suggested that, it is follicle cells, which are responsible for jelly layer accumulation through pinocytosis. Other studies conclude that jelly layer may be produced both externally by follicle cells and internally by the oocytes (Nørrevang, 1968 *cit.* Franklin, 1980; Eckelbarger and Young, 1992; Smiley and Cloney 1985; Smiley 1988; Beijnink *et al.*, 1984).

Microvilli were absent in previtellogenic oocytes where the synthesis rates (yolk and jelly layer accumulation) were relatively low, and then appeared and gradually became complex structures when vitellogenesis commenced. From this, it may be assumed that intensive exocytosis was connected to the change in oocyte outline during development. During vitellogenesis, the oocytes enlarged, and the membrane became involved in intensive material transfer through exocytosis, including jelly accumulation. Such exocytosis may play a role in oocyte enlargement, as the vacuole membrane persisted and was added to the oocyte surface, providing an irregular oocyte outline.

After the oocyte size and jelly thickness reached maximum, synthesis within the cytoplasm and material transfer including exocytosis slowed down or ceased. Simultaneously microvilli became simple and shorter. In addition, stretching during volume increase promoted a smoother oocyte surface. Nonetheless, it was also possible that microvilli developed to anticipate oocyte enlargement, without any correlation with the rate of material transfer.

Oocytes of *B.natans* (Tyler *et al.*, 1994) have also been reported to possess more microvilli during late vitellogenesis. In *S.californicus*, microvilli become more complex, creating microplicae structures (Smiley and Cloney 1985, Smiley 1990), which may be the same structure as the complex microvilli observed in late vitellogenic oocytes of the investigated *H.leucospilota*. Franklin (1980) suggested that shorter microvilli in late vitellogenic oocytes indicate their significance in metabolic activity and supply of material.

Thick oolemma surrounding small oocytes was no longer present when follicle cells started covering oocytes on the way to detach from the tubule lining. Follicle cells appeared to originate from the inner tubule lining, as was also reported from *P.californicus* (Smilley 1988, 1990). Despite similarity in origin, follicle cells in studied population lacked of cilia. Ciliated follicle cells have also been detected in *H.leucospilota* inhabiting Heron Island

(Franklin, 1980) and deepsea holothurian *B.natans* (Tyler *et al.*, 1994). Other structure decorating follicle cells of deepwater holothurians is podocyte. This structure stands on a basal lamina of *B.natans* (Tyler *et al.*, 1992), *Hansenothuria benti* and *Holothuria occidentalis* (Eckelbarger and Young, 1992).

Follicle cells may function as a barrier, protecting oocytes from the surrounding medium. In addition, their presence allowed oocytes to remain in clusters when fresh fecund tubules were dissected. This may have been facilitated by the fact that follicle cells were located between adjacent oocytes. Continuance follicle cells surrounding fullgrown oocytes were also reported in *P.fabricii* (Hamel *et al.*, 1993) and deepwater species such as *Hansenothuria benti* (Tyler *et al.*, 1992) and *Holothuria occidentalis* (Eckelbarger and Young, 1992). However, those in *S.californicus* (Smiley and Cloney, 1985) and *H.leucospilota* from Heron Island (Franklin, 1980) were noted to remain in the tubule lining when oocytes migrated to the lumen.

In addition to creating a physiological microenvironment, follicle cells have been thought to secrete a jelly layer substance and provided nutrition to the oocytes (Kessel 1966; Franklin, 1980; Smiley and Cloney, 1985; Hirai *et al.*, 1973). When Strathmann and Sato (1969) induce oocytes of *P.californicus* with radial nerve extract of starfish, it is revealed that follicle cells produce a maturity inducing substance, which induce germinal vesicle breakdown. Position of follicle cells which remained surrounding fullgrown oocytes until the oocytes reach maximum size, may allow such functions to occur in studied population.

The protuberance connecting oocytes to the follicle shield of the *H.leucospilota* investigated was similar to the structure previously reported as micropyle (Ohshima, 1925) or fibrous cytoplasmic processes (Maruyama, 1980). This may be the only physical bridge maintaining contact between oocyte and follicle cells. In fact, microvilli were never observed reaching the follicle shield. This was also the case in *S.californicus* (Smiley and Cloney, 1985; Smiley, 1988) and other population of *H.leucospilota* at Heron Island (Franklin, 1980).

Previous investigators suggest that protuberance may function: i) as an attachment point for the oocytes to the follicle layer; ii) as a point to which germinal vesicles migrate, and iii) as the area of animal pole at which the polar bodies protrude (Maruyama, 1980; Smiley, 1988, 1990). In *H.leucospilota* inhabiting Heron Island reef (Franklin, 1980), this structure has been suggested to be the point at which young oocytes attach themselves to the tubule lining, and it does not have a physical correlation with follicle shield because the follicle cells remain in the tubule lining when the oocytes migrated to the lumen. However, in this present study, the accumulation of follicle cells at the proximal end of the protuberance may provide evidence for the linkage between follicle shield and the oocyte, and indicated that the follicle cells were connected to each other. This supports the first function of protuberance. Furthermore, protuberance was not located on the same side of nucleus, and did not seem to support the second function. This structure may disappear after the follicle shield was liberated. As only a small number of oocytes (deliberating from excised fecund tubules) with germinal vesicle breakdown were observed, there was insufficient information to clarify this issue.

Ovulation, defined as liberation of oocyte from the somatic cells including follicle cells encircling oocytes (Smiley and Cloney, 1985), did not occur within the ovarian tubules of the population studied. This drew to conclusion that oocyte maturation did not occur within the ovarian tubules of investigated *H.leucospilota*. This supported the suggestion made by Franklin (1980) that oviduct was an important tunnel for maturation. However, it was possible that ovulation and germinal vesicle breakdown occurred just prior to spawning which only occurred during less than 2 week period and was missed in the sampling events. Unfortunately, several spawning experiments conducted during this study were not successful. Other species that exhibits the same condition as the population studied is *P.fabricii* (Hamel *et al.*, 1993), while, another population of *H.leucospilota* (Franklin, 1980) showed slightly different mechanism. Even though maturation e.g. germinal vesicle break down was not reported to occur within the tubules in this population, and sea water did not induce to germinal vesicle break down either, but ovulation does occur within the tubules when oocytes migrate to the lumen and leave the follicle cells in the tubule lining.

Acknowledgement. My gratitude is subjected to Dr. Jim Luong-van and Dr. Michael Guinea for their scientific guidance and encouragement. I am

grateful to my seniors: Dr. A.G. Ilahude, Dr. A. Nontji and Dr. Subagjo who have patiently read the manuscript. I wish to acknowledge AusAID for the financial support for this study.

REFERENCES

Anderson, E. 1974. Comparative aspects of the ultrastrucutre of the female gamete. International Review of Cytology. Suppl. New York Academic Press. Vol.4: 1–70.

Beijnink, F. B., C. W.Walker, and P. A.Voogt. 1984. An ultrastrucutral study of relationships between the ovarian haemal system, follicle cells and primary oocytes in the sea star, *Asteria rubens*. *Cell Tissue Research*, 238, 339–347.

Cooper, E. K. 1985. *Science*. Hardcourt Brace Jovanich, Orlando. 405 pp.

Drosdov, A. L., E. S. Kornienko and G. A. Kryuchkova. 1991. Oocyte maturation: development and metamorphosis of far eastern sea cucumber. *The Soviet Journal of Marine Biology. New York Consultant Bureau*, 16 (6): 347–355.

Eckelbarger, K. J. and C. M.Young. 1992. Ovarian ultrastructure and vitellogenesis in ten species of shallow water and bathyal sea cucumber (Echinodermata: Holothuroidea). *J. Mar. Bio. Ass. UK.*, 72: 795–781.

Franklin, S. E. 1980. The reproductive biology and some aspects of the population ecology of the holothurians *Holothuria leucospilota* (Brandt) and *Stichopus chloronatus* (Brandt). PhD., University of Sydney.250 pp.

Hamel, J.-F., J. H. Himmelman and L. Dufresne. 1993. Gametogenesis and spawning of the sea cucumber *Psolus fabricii* (Buben and Koren). *Biology Bulletin*, 184: 125–143.

Hirai, S., K. Chida and H. Kanatani. 1973. Role of follicle cells in maturation of starfish oocytes. *Dev. Growth and Differentiation*, 15 (1): 21–31.

Holland, N. D. and K. Dan. 1975. Ovulation in echinoderm. *Experentia*, 31: 1078–1080.

Kessel, R. G. 1966. Some observations on the ultrastructure of the oocytes on *Thyone briareus* with special reference to the relationship of the Golgi complex and endoplasmic reticulum in the formation of yolk. *Ultrastructure Research*, 16: 305–319.

Kessel, R.G. 1987. Annulate lamella (porous cytomembrane): with particular emphasis on their possible role in differentiation of female gamete. Development Biology, Comprehensive Synthesis, Plenum Press, New York., 176–233.

Maruyama, Y. K. 1980. Artificial induction of oocyte maturation and development in the sea cucumbers *Holothuria leucospilota* and *Holothuria pardalis*. *Biology Bulletin*, 158: 339–348.

Ohshima, H. 1925. Notes on the development of the sea cucumber, *Thyone briareus*. *Science*, 61, 420–422.

Purwati, P. 2001. Reproduction in *Holothuria leucospilota* Calrk 1920 in the tropical waters of Darwin, NT, Australia. MSc, thesis, Northern Teritorry University. 147 pp.

Purwati, P. and J. T. Luong-van. 2003. Sexual reproduction in a fissiparous holothurian sepcies, *Holothuria leucospilota* Clark 1920 (Echinodermata: Holothuroidea). *SPC Beche-de-mer Info. Bull* 18: 33–37.

Smiley, S. 1988. The dynamics of oogenesis and the annual ovarian cycle of *Stichopus californicus* (Echinodermata: Holothuroidea). *Biology Bulletin*, 175: 79–93.

Smiley, S. 1990. A review of echinoderms oogenesis. *J. Electron Microscopy Technique*, 16: 93–114.

Smiley, S. and R. A. Cloney. 1985. Ovulation and the fine structure of the *Stichopus californicus* (Echinodermata:Holothuroidea) fecund ovarian tubules. *Biology Bulletin*, 169: 342–364.

Strathmann, R. R. and H. Sato. 1969. Increased germinal vesicle breakdown in oocytes of the sea cucumber *Parastichopus californicus* induced by star fish radial nerve extract. *Exp. cell Res.*, 54: 127–129.

Tyler, P. A., K. Eckelberger and D. S. M. Billet. 1994. Reproduction in *Bathyplotes natans* (Holothuroidea: Synaptidae) from bathyal depths in the North-east and Western Atlantic. *J. Mar. Bio. Ass. U.K.*, 74: 383–402.