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Spermatogenesis and chromatin condensation in male germ cells of sea cucumber *Holothuria leucospilota* (Clark, 1920)

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Abstract

Male germ cells in the testis of *Holothuria leucospilota* can be divided into 12 stages based on ultrastructure and patterns of chromatin condensation. The spermatogonium (Sg) is a spherical-shaped cell with a diameter of about 6.5–7 μm. Its nucleus mostly contains euchromatin and small blocks of heterochromatin scattered throughout the nucleus. The nucleolus is prominent. Primary spermatocytes are divided into six stages, i.e., leptotene (LSc), zygotene (ZSc), pachytene (PSc), diplotene (DSc), diakinesis (DiSc) and metaphase (MSc). The early cells are round while in DiSc and in MSc cells are oval in shape. From LSc to MSc, the sizes of cells range from 3.5 to 4 μm. LSc contains large blocks of heterochromatin as a result of increasingly condensed 17 nm fibers. In ZSc, the nucleus contains prominent synaptonemal complexes but a nucleolus is absent. In PSc, heterochromatin blocks are tightly packed together by 26 nm fibers and appeared as large patches in DSc. Heterochromatin patches were enlarged to form chromosomes in DiSc and MSc and then the chromosome are moved to be aligned along equatorial region. The secondary spermatocyte (SSc) is an oval cell about 4.5–5.5 μm. Their nuclei contain large clumps of heterochromatin along the nuclear envelope and in the center nuclear region. Spermatids are divided into two stages, i.e., early spermatid (ESt) and late spermatid (LSt). The nuclei decrease in size by a half and become spherical; thus the chromatin fibers condensed into 20 nm and are closely packed together leaving only small spaces in LSt. The spermatozoa (Sz), with chromatin tightly packed in the spherical nucleus with a diameter of 2 μm and a small acrosome situated at the anterior of the nucleus. The tail consists of a pair of centrioles lying perpendicular to each other and surrounded by a mitochondrial ring, and an axonemal complex, surrounded by a plasma membrane.
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Keywords: *Holothuria leucospilota*; Spermatogenesis; Chromatin; Ultrastructure

1. Introduction

Holothuria leucospilota is widely distributed in the coastal regions throughout the Pacific Ocean (Conand, 1998) in coral reefs, on the sandy sea floor or below rocks in tropical waters up to 3 m deep. *H. leucospilota* is among approximately 20 species that constitute the beche-de-mer fishery in the Indo-Pacific (SPC, 1994).

In holothurians, ultrastructure of spermatogenesis has been reported only in two species, *Cucumaria lubrica* and *Leptosynapta clarki* (Atwood, 1974). Four spermatogenic stages were described: spermatogonia, spermatocytes, spermatids and spermatozoa. However, no studies have been concerned with spermiogenesis, except for those by Pladellorens and Subirana (1975a,b), who briefly mention the condensation of chromatin.

The present study reports that ultrastructural observations were made on spermatogenesis and spermiogenesis in *H. leucospilota*, particularly on the process of chromatin condensation in the nucleus.

2. Materials and methods

A total of 12 adult males of *H. leucospilota* (25–30 cm length) were collected every two months during October 2003 to October 2004 from the coastal water of the eastern

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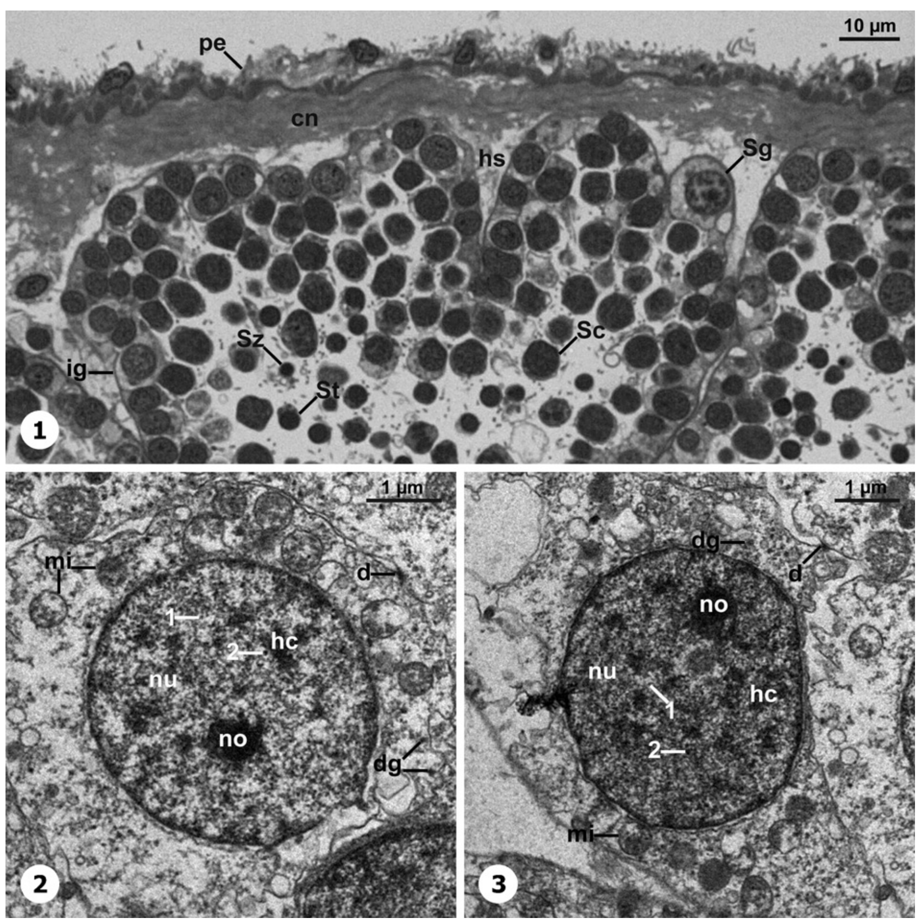
Gulf of Thailand, Satahip, Chonburi province, Thailand, transported to the laboratory, and maintained in aquaria with a natural photoperiod. Observations were made on fresh gonadal tubules. Small portions of tubules were fixed in a mixture of 4% glutaraldehyde and 2% paraformaldehyde in 0.1 M sodium cacodylate buffer (pH 7.8) for 12 h at 4 °C and postfixed in 1% osmium tetroxide in the same buffer for 1 h. After double fixation, the sample was treated for transmission electron microscopy (TEM) and scanning electron microscopy (SEM). For TEM, samples were dehydrated in a grade ethyl alcohol series, followed by two changes of acetone, and embedded in low viscosity Spurr resin. Semithin sections were cut on a Reichert ultramicrotome with a glass knife, and stained with 1% methylene blue. Ultra-thin sections were also made on an ultramicrotome and stained with uranyl acetate and lead citrate. Sections were examined under a Philips TECHNAI 20 transmission electron microscope operating at 75 kV. The width of chromatin fibers was measured from the electron micrographs negatives using catalase crystal with the lattice spacing 8.75 nm as the standard. For SEM, samples were attached to coverslips previously coated with 0.1% poly-L lysine. Samples were fixed and dehydrated as described above, then critical-point dried and gold coated. Preparations were examined in a LEO 1450 VP scanning electron microscope.

3. Results

3.1. Histology of the testis

The gonad of *H. leucospilota* is composed of branching gonadal tubules joined together by a single gonoduct. Each gonadal tubule consists of three layers of tissues (Fig. 1).



Figs. 1–3. (Fig. 1) *Holothuria leucospilota* gonadal tubule showing the three layers of tissues. The outer most is peritoneal epithelium (pe). In the middle is a loose network of connective tissue (cn). The innermost is invaginated germinal epithelium (ig). The haemal sinus (hs) is situated between the connective tissue and the germinal epithelium. Different stages of germ cells are distributed in the gonadal tubule, i.e., spermatogonia (Sg), spermatocyte (Sc), spermatid (St), spermatozoa (Sz). (Fig. 2) *H. leucospilota* spermatogonia (Sg) contain prominent nucleolus (no) and circular nucleus (nu). Patches of heterochromatin (hc) distribute around the nucleus and a thin stripped chromatin lie along the nuclear envelope. The chromatin fibers consist of two levels: 7 nm fibers (1) and 17 nm fibers (2). The cytoplasm contains numerous small round mitochondria (mi) and dense granule (dg). The spermotogonia are connected to each other by the desmosome-like structure (d). (Fig. 3) *H. leucospilota* leptotene primary spermatocyte (LSc) showing the circular nucleus with prominent nucleolus (no). Numerous of small patches heterochromatin (hc) distribute throughout the central nuclear region and lie close contact to the nuclear envelope. The heterochromatin exhibit 2 levels: 7 nm and 17 nm fibers. The cytoplasm contains a number of small round mitochondria (mi) and numerous of small dense granules (dg). Each leptotene primary spermatocyte connects to each other by desmosome-like structure (d).

The outermost is thick peritoneal epithelium. The middle layer is a loose network of connective tissue. The innermost layer (germinal epithelium) forms several deep invaginations throughout the length of the gonadal tubule. The germinal epithelium contains germ cells in various stages of development.

3.2. Classification of spermatogenic cells

Male germ cells were classified into 12 stages based upon cell sizes, morphology and size of nuclei, their structural features and chromatin patterns.

The spermatogonium is a spherical shaped cell with a diameter of 6.5–7 μm. Its nucleus is round with a diameter 4–4.5 μm. The nucleolus is distinct in the central area of the nucleus. The nucleus contains a thin band of heterochromatin that attaches to the inner surface of the nuclear membrane and small patches of heterochromatin are distributed throughout the nucleus. Chromatin fibers consist of two levels, i.e., 7 and 17 nm fibers. The former appear as thin zigzag lines, while the latter appear in cross section as dense dots. The heterochromatin is formed by aggregations of the 17 nm fibers. The cytoplasm contains numerous dense granules and small round mitochondria. Most of the mitochondria concentrate at one side of the cell (Fig. 2).

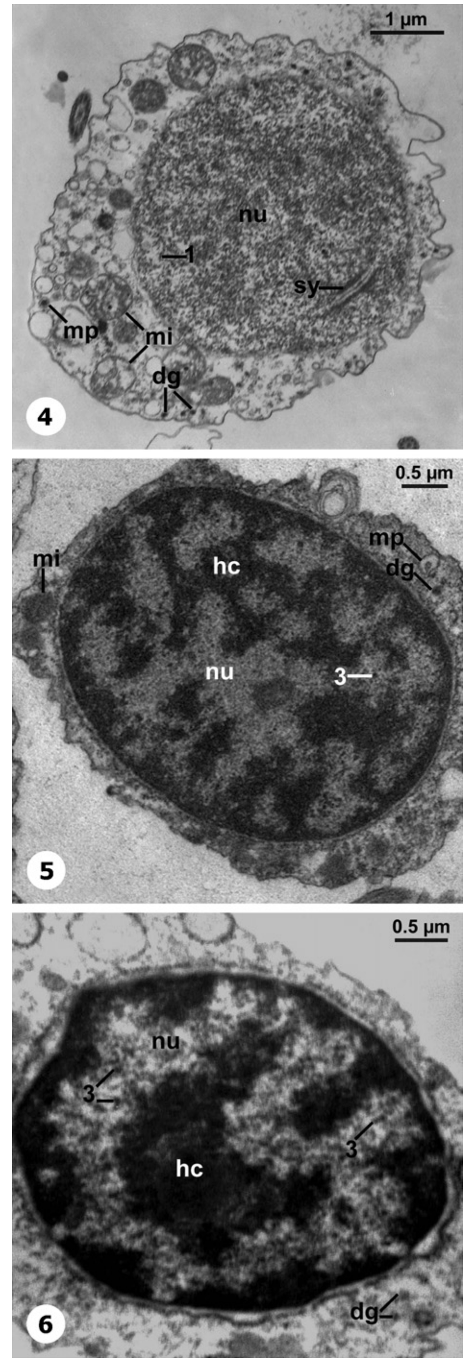
3.2.1. Primary spermatocytes

Primary spermatocytes are composed of six stages, i.e., leptotene, zygotene, pachytene, diplotene, diakinetic and metaphase. The most distinctive difference among various stages of primary spermatocytes is the patterns of chromatin condensation.

Leptotene spermatocyte: This round cell is smaller than spermatogonia with a diameter of 4.5–5.5 μm and contains a round to oval nucleus with a diameter 3.7–4 μm. The nucleus appears denser and the amount of heterochromatin increases greatly in comparison to spermatogonia. Most of heterochromatin fibers are connected to each other side by side and also connected to the heterochromatin strip at the inner surface of the nuclear envelope. The chromatin fibers still consist of two levels as in spermatogonia, but the 17 nm fibers are greater in number. The nucleolus is still present (Fig. 3). The cytoplasm has similar features to spermatogonia.

Zygotene spermatocyte: This cell still has a round shape and is nearly the same size as leptotene spermatocyte. The distinguishing features of zygotene spermatocyte is the density of heterochromatin which is scattered throughout the circular nucleus. Chromatin fibers exhibit only 26 nm fibers. Within the nucleus, there is the first evidence of synaptonemal complexes. The cytoplasm has similar characteristic as leptotene spermatocyte. The Golgi complex is found nearly to the groups of small ovoid mitochondria and also possesses numerous membrane-bound proacrosomal granules segregate in close association with its cisternae (Fig. 4).

Pachytene spermatocyte: This cell is oval in shape with a diameter of 4–5 and 3.5–4 μm in nuclear diame-



Figs. 4–6. (Fig. 4) *H. leucospilota* zygotene primary spermatocyte (ZSc) showing the circular nucleus (nu), with increasingly condensed heterochromatin and presence of synaptonemal complexes (sy). The chromatin fibers appear in 7 nm in diameter (1). Cytoplasm consists of abundant ovoid mitochondria (mi), many dense granules (dg), and membranous-bound proacrosomal granules (mp). (Fig. 5) *H. leucospilota* pachytene primary spermatocyte (PSc) showing oval nuclei (nu) which contain large heterochromatin blocks (hc) which interconnect. Individual chromatin condenses in to the size of 26 nm (3) in diameter. Cytoplasm consists of round mitochondria (mi), membranous-bound proacrosomal granules (mp), and numerous of small dense granules (dg). (Fig. 6) *H. leucospilota* diplotene primary spermatocyte (DSc). The nucleus (nu) contains large patches heterochromatin (hc) within the central region and lying along the nuclear envelope. Chromatin fibers are about 26 nm in diameter (3), but are more tightly packed than in the PSc stage. The cytoplasm contains numerous dense granules (dg).

ter. The heterochromatin appears entwined as the result of interconnections between adjacent heterochromatin and the attachment of heterochromatin to some area of the nuclear envelope. Individual chromatin fibers in the heterochromatin cords are enlarged and appear as 26 nm tightly packed fibers (Fig. 5). The cytoplasm has similar features to zygotene spermatocyte.

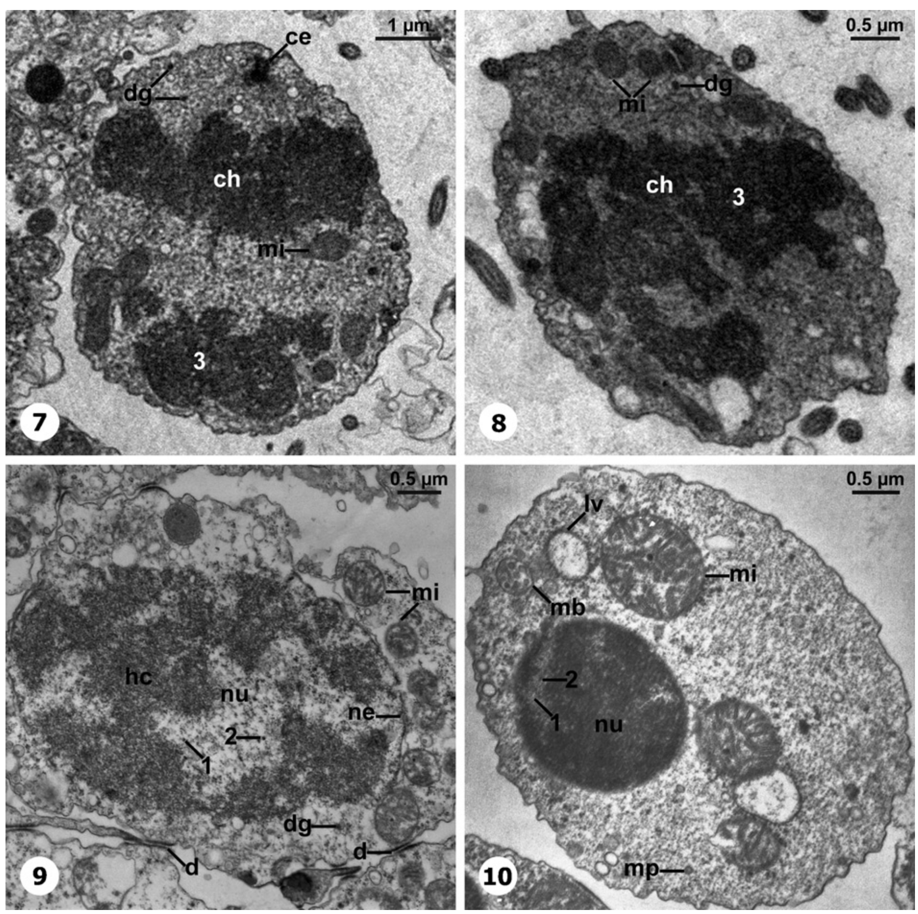
Diplotene spermatocyte: This cell is approximately the same size as pachytene spermatocyte. The distinguishing feature of diplotene spermatocyte is the increasing condensed of heterochromatin and the tightly packed 26 nm fibers (Fig. 6).

Diakinetic and metaphase spermatocytes: Cells in these stages are about 4.5–5.5 in size and contain large chromatin blocks consisting of tightly packed 26 nm fibers that are part of complete forms of chromosome in diakinetic stage (Fig. 7), which are separated and then move to be aligned at the equatorial region in metaphase (Fig. 8). Centrioles appeared at either side of the cells. Cytoplasm contains ovoid mitochondria, rough endoplasmic reticulum, and numerous dense granules, all dispersed throughout the cytoplasm.

Secondary spermatocyte is an oval cell of about 4.5–5.5 μm with a nucleus of about 3.5–4 μm in diameter. The nucleus shows large clumps of coarse dense chromatin that arise along the nuclear envelope and passes the central nuclear region. Chromatin fibers consist of 7 and 17 nm fibers. The 17 nm fibers are aggregated to form heterochromatin, while the 7 nm fibers are scattered in the euchromatic regions. Cytoplasm consists of numerous ovoid mitochondria which are concentrated at one side of the cells (Fig. 9).

3.2.2. Spermiogenic cells and spermatozoa

There are two stages of spermatids: spermatid I and spermatid II, depending on nuclear size, formation of acro-



Figs. 7–10. (Fig. 7) *H. leucospilota* diakinesis primary spermatocyte (DiSc) showing thick heterochromatin blocks that are parts of the completely formed chromosomes (ch). The chromosomes consisting of tight aggregations of 26 nm fibers (3). Centrioles (ce) are present at one side of the cell. Numerous dense granules (dg) are scattered throughout the cytoplasm. (Fig. 8) *H. leucospilota* metaphase primary spermatocyte (MSc). The nucleus contains pieces of chromosomes (ch) which appear in 26 nm in diameter (3). Ovoid mitochondria (mi) and dense granule (dg) are distributed throughout the cytoplasm. (Fig. 9) *H. leucospilota* secondary spermatocyte (SSc) contain an oval nucleus (nu) with the large clumps of heterochromatin (hc) arising from the nuclear envelope (ne) and passing through the central nuclear region. Chromatin fibers consist of two levels; 7 nm fibers (1) and 17 nm fibers (2). The cytoplasm consists of numerous ovoid mitochondria (mi) and dense granules concentrated at one side of the cell. d: desmosome-like structure. (Fig. 10) *H. leucospilota* spermatid I (St_1) showing small nuclei with large clumps of heterochromatin (hc) packed together. Chromatin fibers consist of two levels: 7 nm fibers (1) and 17 nm fibers (2). Ovoid mitochondria (mi) in the cytoplasm are reduced in number and larger in size. Other cytoplasmic organelles, such as membrane-bound proacrosomal granules (mp) and the large electron-lucent vesicle (lv) are also shown.

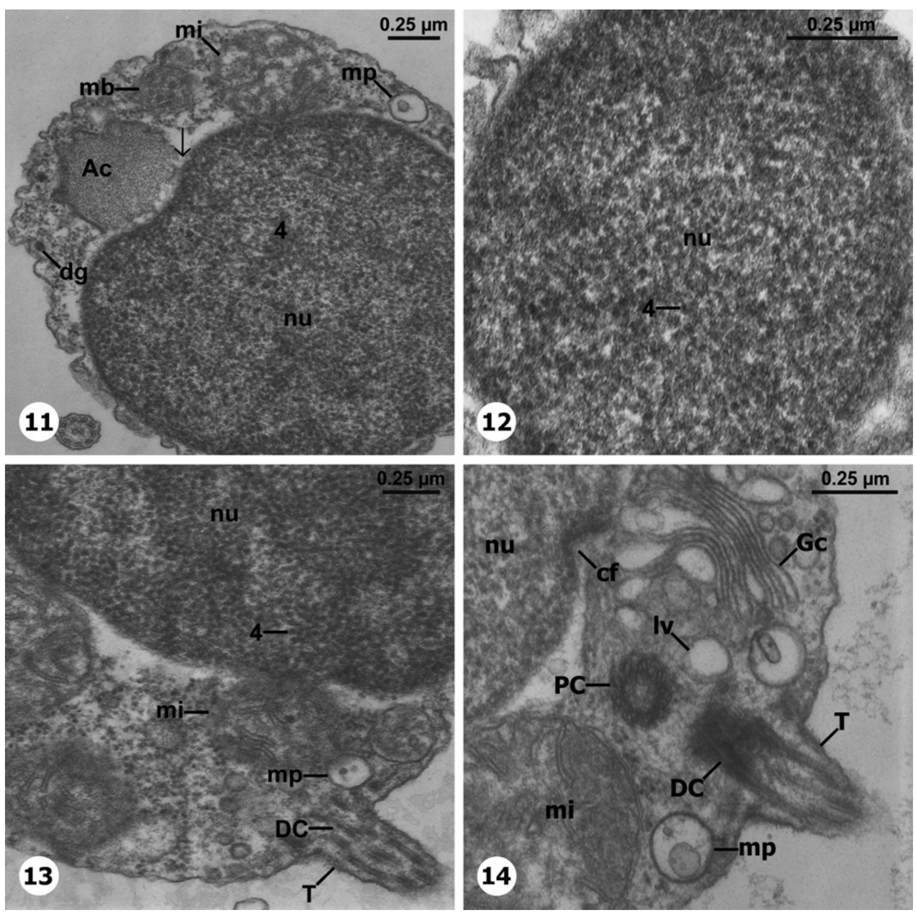
somal vesicle and flagella, and the pattern of chromatin condensation.

Spermatid I: This cell still is oval shaped with slightly smaller size than secondary spermatocyte. The cell possesses a spherical nucleus (2.5–4 μm in nuclear diameter) with large clumps of heterochromatin distributed in the central nuclear region as well as along the nuclear envelope. Within the cytoplasm are accumulated mitochondria, numerous of the membrane-bound proacrosomal granules, large multigranular body comprising of six to eight small dense granules, and the large electron-lucent vesicles (Fig. 10).

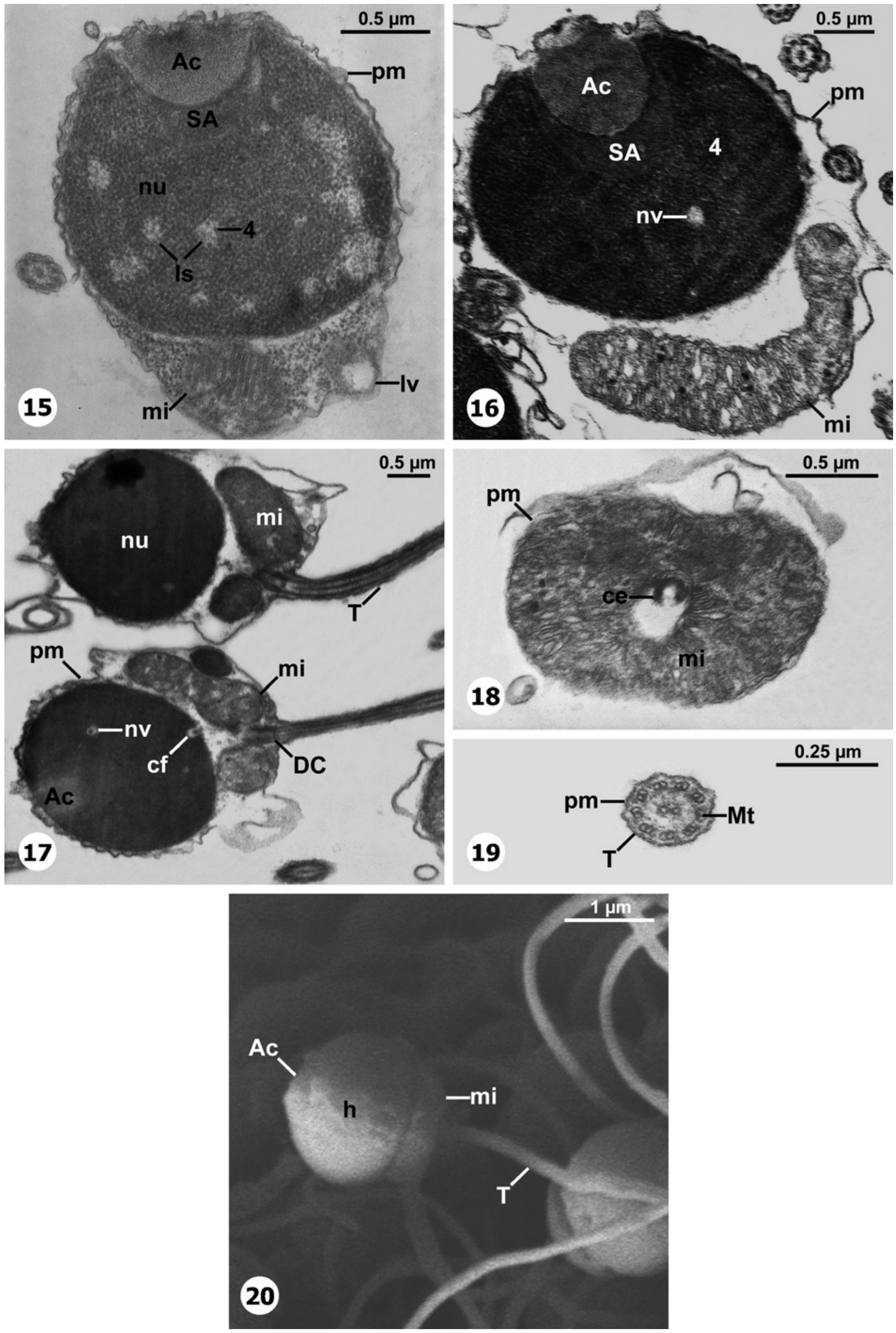
Spermatid II: This cell has more spherical shape than that of the spermatid I stage. The nucleus has become circular (2 μm in diameter), and is located eccentrically within the cell. The heterochromatin is condensed into 20 nm fibers (level 4) and appears in cross sections as coarse dense granules (Figs. 11 and 12). The membrane-bounded proacrosomal granules and the dense granules which are observed in the previous stages migrate to the apex of the cell and subsequently invaginated by the nucleus (Fig. 11), result in the formation of acrosomal granule (Fig. 11). Within the cytoplasm, the large mitochondria migrate to lie at the base of the nucleus (Fig. 13). A pair of centrioles is situated near the mitochondria. The two centrioles lie perpendicular to each other (Fig. 14). The stacks of the Golgi complex are observed near to the centrioles and the mitochondria. Dense membrane-bound proacrosomal granules become scarce while the electron-lucent vesicles increase in number (Fig. 14).

Spermatozoa: There are two stages of spermatozoa: immature spermatozoa and mature spermatozoa.

Immature spermatozoa possess the circular head (2 μm in diameter). All 20 nm chromatin fibers are tightly packed, so the interchromatin spaces occur in the nucleus (Fig. 15). At



Figs. 11–14. (Fig. 11) *H. leucospilota* spermatid II (St_2) with the nucleus (nu) contains coarse dense chromatin fibers and a single electron-opaque acrosomal granule (Ac). The chromatin appear in uniformly size as the 20 nm fibers (4). The nucleus exhibits an intermediate region (arrow) that will later invaginate to contain the periacrosomal material. This cell contains slightly cytoplasm which consists of few mitochondria (mi), the membrane-bound proacrosomal granules (mp), the multigranular body (mb), and numerous dense granules (dg). (Fig. 12) *H. leucospilota* spermatid II (St_2). Higher magnification of a longitudinal section through the nucleus (nu). The nucleus contains chromatin fibers which appear as coarse dense granules. The chromatin appear uniform in size as the 20 nm fiber (4) distributed throughout the nucleus. (Fig. 13) Cytoplasm region of *H. leucospilota* spermatid II that contains only two to three large ovoid mitochondria (mi) lie at the base of nucleus. The tail (T) is extending from the distal centriole (DC) in the acute angle to the nuclear longitudinal axis. Mp: membrane-bound proacrosomal granule. (Fig. 14) *H. leucospilota* spermatid II (St_2) showing highly packed heterochromatin 20 nm fibers (4) in the nucleus (nu) surround by a thin plasma membrane. Within the cytoplasm, the two mitochondria are fused to form a large single mitochondrion (mi). The membrane-bound proacrosomal granules (mp) become scarce and appear in less electron-densed than in the previous stages. The electron-lucent vesicles (lv) are increasing in number. A pair of centrioles lying perpendicular to each other with the proximal centriole (PC) situates near the nucleus and the distal centriole (DC) protruding to form the tail.



Figs. 15–20. (Fig. 15) *H. leucospilota* immature spermatozoon showing a subspheroidal nucleus (nu) surrounded by a thin serrated plasma membrane (pm). Heterochromatin is highly condensed with interchromatin spaces (Is). Individual chromatin fibers have an average diameter of 20 nm in diameter (4). At the anterior end is the acrosome (Ac) situated above the subacrosome (SA). Cytoplasm at the posterior end of the nucleus contains a single mitochondrion (mi) and a large electron-lucent vesicle (lv). (Figs. 16 and 17) *H. leucospilota* mature spermatozoon showing the circular nucleus (nu) surrounded by a thin rim of plasma membrane (pm). Heterochromatin is condensed and only one nuclear vacuole (nv) is present. Each chromatin fiber is about 20 nm in diameter (4). The spherical acrosome (as shown in cross section) is located at the anterior of the cell above the subacrosome (SA). Nuclear vacuole is present. The shallow centriolar fossa (cf) is formed. The large mitochondria ring (mi) houses the centriole (DC). Sperm tail (T) is very long. (Fig. 18) A cross section of a sperm tail (T) through the mitochondrial ring (mi) and centriole (ce). pm: plasma membrane. (Fig. 19) Cross section of distal end of the sperm tail (T) showing the 9 + 2 microtubule (Mt) pattern of the axonemal complex surrounded by a thin plasma membrane (pm). (Fig. 20) Scanning electron micrograph of a spermatozoon (Sz) showing the subspheroidal head (h) situated anteriorly by a small spherical acrosome (Ac). A mitochondrial ring (mi) lies at the base of the nucleus and separates the head (h) from the long tail (T).

Table 1
Dimension of chromatin fibers in the nuclei of stages of male germ cells in the gonadal tubules of *H. leucospilota*. Values are expressed as mean ± S.D.

Male germ cells of *H. leucospilota*	*N*	Diameter of chromatin fibers (nm)			
		Level 1	Level 2	Level 3	Level 4
Spermatogonia	20	6.68 ± 0.22	16.51 ± 0.34	–	–
Leptotene spermatocyte	20	6.73 ± 0.34	16.91 ± 1.34	–	–
Zygotene spermatocyte	20	6.69 ± 0.74	–	–	–
Pachytene spermatocyte	20	–	–	26.47 ± 4.23	–
Diplotene spermatocyte	20	–	–	6.63 ± 1.29	–
Diakinesis spermatocyte	20	–	–	26.49 ± 1.34	–
Metaphase spermatocyte	20	–	–	26.50 ± 1.34	–
Secondary spermatocyte	20	7.38 ± 1.22	17.14 ± 3.00	–	–
Spermatid I	20	6.98 ± 0.27	16.59 ± 0.66	–	–
Spermatid II	20	–	–	–	20.08 ± 0.09
Immature spermatozoa	20	–	–	–	19.92 ± 0.35
Mature spermatozoa	20	–	–	–	20.56 ± 0.11

the anterior end, the nucleus is invaginated and completely forms a subacrosome embedded in the deep cup-shaped nuclear fossa. An acrosome is situated above the subacrosome. The acrosome is surrounded externally by a serrated plasma membrane extending to the lateral side of the nucleus (Fig. 15). The basal cytoplasmic area is composed of a large mitochondria ring. Numerous electron-lucent vesicles usually appear to lie close to many points of the plasma membrane (Fig. 15).

The mature spermatozoon is a spheroidal-shaped cell about 2 μm in diameter. Its chromatin fibers have already reached their highest condensation. Chromatin fibers remain 20 nm in diameter and are arranged in irregular patterns (Fig. 16). There is an acrosome with cup-shaped subacrosome apposing on the anterior tip of head. The acrosome is surrounded externally by a serrated plasma membrane extending to the lateral side of the nucleus (Fig. 16). The tail is long with a pair of centrioles surrounded by a large mitochondria at the neck region from which the axonemal microtubules is generated (Figs. 17 and 18). The axoneme has the classic 9 + 2 microtubular doublet construction (Fig. 19). The external morphology of the spermatozoon under SEM reveals that it consists of a round head (2 μm in diameter) with a rough surface. A small spherical acrosome is situated at the anterior end of the head. At the posterior end of the head, there is a large mitochondrial ring which separates the head from a long tail (Fig. 20; Table 1).

4. Discussion

4.1. Testicular histology and classification of cells in spermatogenesis

The gonadal tubules of *H. leucospilota* have completely limiting epithelium, which is divided into three layers: a thick peritoneal epithelium, a loose network of connective tissues, and a germinal epithelium. Similar histology studies in other species were performed by other investigators (Drozdov et al., 1986; Reunov et al., 1994; Hamel et al., 1993). The most recent studies have not been rigorously categorized the various stage of spermatogenesis in *Holothuria*, apart from two species of sea cucumbers, *C. lubrica* and *L. clarki* in which there are four classes; i.e., spermatogonia, spermatocytes, spermatids and spermatozoa (Atwood, 1974). In the present study, the process in *H. leucospilota* could be classified into twelve stages according to size, shape, cytoplasmic features and pattern of chromatin condensation. Spermatogonia of *H. leucospilota* are interconnected by a desmosome-like structure similar to that in the sea cucumbers, *C. lubrica* and *L. clarki* and in sea urchins *Arbacia punctulata* and *Strongylocentrotus purpuratus* (Longo and Anderson, 1969). The spermatogonium gives rise to primary spermatocytes which pass through six stages as in the first meiotic division as in vertebrate germ cells (Courot et al., 1970). In *H. leucospilota*, the primary spermatocytes remained at nearly the same size as that of spermatogonia. However, nuclei exhibit further changes in degree of chromatin condensation. In leptotene primary spermatocyte, heterochromatin patches appear in the nucleus in larger numbers than in spermatogonia. However, chromatin still appears in two levels; 7 and 17 nm fibers, which correspond to levels 1 and 2, respectively. In zygotene primary spermatocytes, chromatin fibers exhibit only 7 nm fibers; it is conceivable that the condensed 17 nm fibers are decondensed 7 nm and are active in transcriptional activity. The decondensation of chromatin fibers into the active state results in the production of various amounts and types of mRNA. This also relates to the presence of numerous dense granules and membrane-bound acrosomal granules in the cytoplasm. In pachytene spermatocyte of *H. leucospilota*, chromatin fibers appear in cross section as large dense dots measuring about 26 nm (level 3) that may be interpreted as highly condensed 7 nm coiled chromatin fibers. The chromatin fibers in the heterochromatin blocks appear as the 26 nm type and are increasingly packed from diplotene spermatocyte to metaphase spermatocyte stage. After the first meiotic division is completed, the daughter cells arising from this division are secondary spermatocytes. This stage is rarely encountered in the gonadal tubules sections of *H. leucospilota*. The implied short transit time of secondary spermatocyte is also reported in higher vertebrates (Dym and Fawcett, 1971; Chavadej et al., 2000). The secondary spermatocyte of *H. leucospilota* is still connected to each other by desmosome-like structures which completely disappear after this stage. This implies that the cells from spermatogonial stage to the secondary spermatocyte stage differentiate synchronously in accordance with that in other higher vertebrates (Clermont, 1966; Kalt, 1976; Andrade et al., 2001).

4.2. Cells in spermiogenesis and formation of spermatozoa

Two stages of spermatid development could be identified in *H. leucospilota*, based on nuclear size, shape and condensation of chromatin pattern. The nuclei maintain a circular conformation throughout spermiogenesis and remain unchanged in the mature spermatozoa. The centriole is not invaginated. It presents one of the primitive types of spermiogenesis. The two stages of spermatids exhibit finely dense chromatin fibers. The chromatin fibers appear in two levels; the 7 nm fibers (level 1) disperse in the light nucleoplasm and the 17 mm fibers (level 2) condense into the large clumps of heterochromatin. The chromatin fibers found in the late stages of primary spermatoctye are recognized again in secondary spermatocyte and spermatid I stage. From spermatid II stage, the nucleus contains chromatin that condenses into highly coiled fibers of 20 nm (level 4), a size very similar to that found in somatic nuclei. The limited degree of condensation in chromatin found in *H. leucospilot*a may also be an indication that histones are still conserved and are not replaced by protamine or other proteins as found in *Limulus polyphemus* (Fahrenbach, 1973).

Our studies have shown that synthesis of proacrosomal granule is initiated early in the spermatogonial stage in contrast to higher invertebrate such as the giant African snail *A. fulica* (Pankao, 2000) and hamster (Miething, 1998). In the cytoplasm of spermatogonia, numerous dense granules are widely distributed. Soon after in the zygotene spermatocyte, the large single Golgi complex appears and numerous membrane-bound proacrosomal granules are situated in closed association with the Golgi complex cisternae. It seems that the small dense granules in spermatogonia are the precursors of the proacrosomal granules which are fused together into various sizes of membrane-bound proacrosomal granules in zygotene spermatocyte. Both the small dense granules and the membrane-bound proacrosomal granules are present in the cytoplasm through all stages of primary spermatocytes and secondary spermatocytes. They will contribute to formation of the acrosomal granules in the spermatid II stage. The acrosome is fully forming in the spermatid II where it appears in the shape of a deep cup. This structure is similar to that found in starfish and sea urchin (Hodgson and Bernard, 1986; Fontaine and Lambert, 1976). The acrosome contains a homogenous matrix which is not reported in other sea cucumbers (Dan, 1960). The characteristics of *H.* leucospilota spermatozoa followed the primitive sperm type with a spheroidal nucleus as in the spermatozoa of other holothurians (Hodgson and Bernard, 1986; Fontaine and Lambert, 1976; Pladellorens and Subirana, 1975a,b). On the other hand, in some species of sea cucumbers, such as *C. lubrica* (Atwood, 1974) and *C. pseudocurata* (Atwood, 1975), the spermatids nuclei undergo a process of nuclear elongation during spermiogenesis resulting in a conical shaped head in the mature spermatozoon. Other similarities with starfish and sea urchin can be found in the presence of a single mitochondrial ring.

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