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Original Article

Electron microscopy reveals the ultrastructure and the chromatin organization of *Holothuria scabra* male germ cells

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Abstract

Holothuria scabra release their gametes into seawater where external fertilization rapidly takes place. *H. scabra* sperm are generated through the steps of germ cell proliferation and differentiation within the wall of the testicular tubule. The outer most layer of the tubular wall supports the developing germ cells. All germ cells are classified according to the hetero-chromatization pattern of the cell nuclei. Based on the degrees of chromatin condensation, the two spermatid stages are classified as St1 and St2. The St1 exhibits a small, round nucleus with heterochromatin clumping around the nuclear envelope which, in turn, is surrounded by the mitochondrial aggregation. In the St2, thick chromatin fibers appear. Large mitochondria fuse and the tail rudiment also starts to form at the posterior, whereas acrosome and subacrosomal structures are fully formed at the anterior region. It is speculated that a coiling of nucleosomes organized by the sperm-specific histones causes the chromatin condensation in spermatids and sperm nuclei of *H. scabra*, much like what happens in sea urchin sperm which indicates a highly conserved phylogenetic process. The appearance of numerous phagosomes in the Sertoli-like cells suggests their possible roles in providing nutrients to the proliferating germ cells and phagocytic removal of cytoplasmic debris from the maturing germ cells.

Keywords:sea cucumber, *Holothuria scabra*, reproduction, spermatogenesis, chromatin condensation

1. Introduction

The sea cucumber, *Holothuria scabra*, is one of the most commercialized species favored by local consumers as well as for exports around Asia. The animal is found in shallow water of the tropical coastal areas of the Indo-Pacific region (Conand, 1998). *H. scabra* belongs to the phylum Echinodermata and is closely related to the sea urchins and the sea stars. These animals exhibit a bilateral (ventral-dorsal) symmetry of the body and a radial symmetry of the nerve ring.Like other marine animals, the sea cucumber reproduces bysynchronous broadcast spawning of gametes into the seawater where sperm make direct contact with eggs to begin the process of fertilization.

H. scabra is a dimorphic organism with separate male and female sexes. Based on histological observation and maturity index (Ramofafia, Byrne, & Battaglene, 2003; Rasolofonirina, Vaïtilingon, Eeckhaut, & Jangoux, 2005), the maturation of the testis has been classified into five continuous phases: the spent, the recovery, the growing, the

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maturation, and the spawning phases. These cyclical phases appear year-round with a synchronous development of testicular branches corresponding to their gametogenic cycles (Sewell, Tyler, Young, & Conand, 1997).

The general morphology of mature sperm is quite similar among species of echinoderms, such as a spherical head with a small acrosome and a motile flagellum, except for the sea urchin sperm whose long, conical-shaped head is distinctive from the others (Walker, Unuma, & Lesser, 2013). Until now, there are only a few reports on spermatogenesis of the sea cucumber, particularly in *H. scabra*, in comparison to the sea urchins and the sea stars which have been studied extensively with regards to structural and biochemical changes during the entire process of spermatogenesis (Demeuldre & Eeckhaut, 2012;Summers, Hylander, Colwin, & Colwin, 1975; Walker *et al.*, 2013). The present study, therefore, focused on investigating the morphological changes in the *H. scabra* male germ cells during their differentiation in the testicular tubules, especially with regards to the condensation pattern of nuclear chromatin and the changes of cytoplasmic organelles.

2. Materials and Methods

Adult male sea cucumber, *Holothuria scabra* (22-26-cm long, weighing around 250-300g) were captured from the coastal area of the Andaman Sea, Southern Thailand, during the seasonal breeding periods (April-June and September-November). The animals were anesthetized by 3% magnesium chloride in seawater prior to dissection. The ventral surface of the body wall was cut open to reveal a coelomic (peritoneal) cavity and visceral organs. Testes were removed and imme-diately placed in a fixative solution (2.5% glutaraldehyde, 4% paraformaldehyde in phosphate buffer, pH 7.2) for overnight at 4ºC. Small pieces of the testicular tubules were chopped and placed in 1% osmium tetroxide for 1 hr at 4ºC, followed by several washes in the buffer. The tissue blocks were then processed by routine preparation for transmission electron microscopy (TEM) by embedding in Araldite 502 resin. In order to observe a large area of the germinal epithelium which makes up the tubular wall, the germ cell characteristics and their organization, semithin sections of the testis were stained with methylene blue and basic fuchsin dyes and then examined by a light microscope (Nikon Eclipse E600). For ultrastructural investigation, thin sections of the testes were cut from the same block by an ultramicrotome (Leica EM UC6). A 300-mesh copper grid containing thin sections of the testicular tubules was stained with uranyl acetate and lead citrate and observed by an electron microscope (Hitachi H-7100 TEM, Hitachi Co. Ltd., Japan) operating at 100 kV.

3. Results

3.1 External morphology of the testis

Mature adult male *H. scabra* exhibited a dark gray thick skin on the dorsal surface and the ventral surface had a white thinner skin (Figure 1A). Dissection through the ventral surface revealed visceral organs and the creamy testis, which was located at the anterior-ventral compartment connected partly to the upper respiratory tree and the upper digestive tract. The testis was composed of several tubules connected to each other at the proximal end to form the large common duct. Each primary testicular tubule branched twice to form secondary and tertiary tubules. Along its entire length, each testicular tubule showed a saccule-like or a segmented feature. All testicular tubules converged to the main stalk at the top which later merged to become the main duct (Figure 1B). The main duct opened directly to the external environment via a small pore located underneath the mouth. Sperm were released through this pore during spawning.

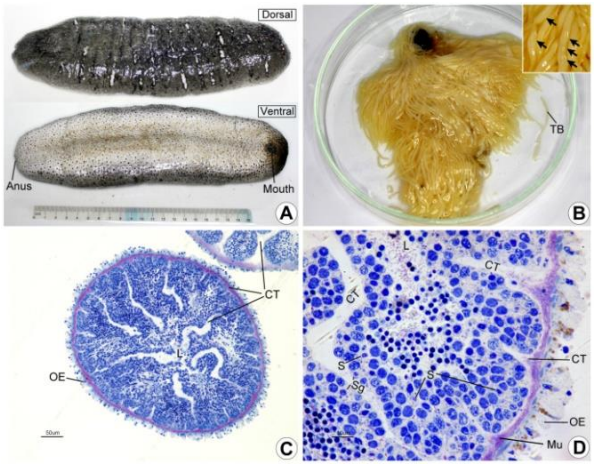

Figure 1. Adult male *H. scabra* showing the white ventral and dark gray dorsal surfaces (A). The testis located at the anterior end of the body was dissected out and displayed. It is composed of several tubules converging together at the testicular base that contains opening of the main duct near the mouth (B). Note a saccule-like feature of the testicular tubule (arrows in B-inset). A semithin section of the testicular tubule stained with methylene blue shows the cell nuclei and basic fuchsin showing the connective tissues (C). A magnified image of the testicular tubule showing the outer epithelial cells lining the tubular wall externally and connective tissue layer that also branches to form trabeculae into the germinal epithelium.
CT, connective tissue; L-lumen; OE, outer epithelium; S, Sertoli-like cell; Sg, spermatogonium; TB, testicular tubule.

3.2 Histology of the testicular tubule

A testicular tubule in cross section exhibited a round, dense circular structure with a diameter of 400-500 µm. The wall of each tubule consisted of four distinctive layers of cellular associations: the outer epithelial cells; the muscular layer; the connective tissue layer; and the germinal epithetlium. The semithin section of the tubule stained with methylene blue and basic fuchsin demonstrated the cell nuclei appearing in blue, while the connective tissues appearing in magenta (Figures 1C, 1D). The outer epithelium lining the periphery of the testicular tubule was composed of at least two types of long columnar cells: the large vacuolated cells and the small pigmented cells (Figures 1D, 2A). Under a thin basal lamina supporting the outer epithelium is a single layer of muscle cells. Interior to this layer is thick connective tissue about 5 µm in width. This layer also branched off as trabeculae stretching inwards to the central lumen dividing the germinal epithelium into columns (Figures 1C, 1D, 2B).

Ultrastructurally, components of the connective tissues constituting the tubular wall and the invaginated trabeculae were similar (Figures 2A, 2B). However, in the tubular wall the abundant collagen and elastic fibers were densely packed, while they were loosely organized in the connective tissues of the invaginated trabeculae. Another thicker basal lamina was present next to the connective tissue layer that supported the germinal epithelium. Early stage developing germ cells, including spermatogonia and early spermatocytes, were resting on this basal lamina and the connective tissue layer (Figures 2B, 2C). Elongating and branching nurse cells, probably equivalent to Sertoli (S) cells in vertebrate testes, were located in between early germ cells and spermatids (Figure 2D). The S cell has a large nucleus that typically contained darkly stained patches of heterochromatin along the nuclear envelop and in the central area (Figure 2D). There were many large vesicles of lysosomes or phagosomes in the cytoplasm. Cytoplasmic processes of the S cells extended to embrace germ cells nearby.

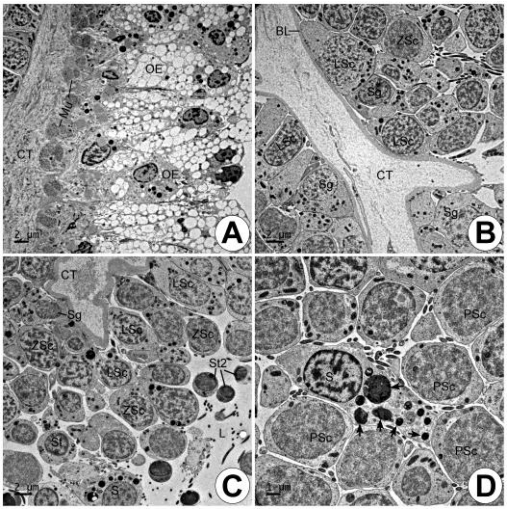

Figure 2. Low magnification TEM micrograph demonstrates the tubular wall containing dense collagenous connective tissue (CT), a muscular layer (Mu) and an outer epithelium (OE) (A). Proliferating early germ cells resting on the thin basal lamina supported by connective tissue trabeculae (B). Sertoli-like cell within the germinal epithelium exhibits dense bands of heterochromatin along the nuclear envelope and in the central area of the nucleus, numerous lysosomal and autophagolysosome vesicles (arrows) in the cytoplasmic processes (C, D).
BL, basal lamina; CT, connective tissue; L, lumen; LSc, leptotene spermatocyte; Mu, muscle; OE, outer epithelium; PSc, pachytene spermatocyte; S, Sertoli-like cell; Sg, spermatogonium; St, spermatid; ZSc, zygotene spermatocyte.

3.3 Ultrastructure of the male germ cells

In a semithin section of the testicular tubule, only a single pattern of cellular association was found. Spermatogonia and some early spermatocytes were resting on the basal lamina next to the connective tissue layers of both the tubular wall and the invaginated trabeculae (Figures 1D, 2B, 2C). Spermatogonia apparently divided and gave rise to primary and secondary spermatocytes which move to the next adluminal layer while being surrounded by the cytoplasmic processes of the S cells. Spermatids derived from secondary spermatocytes were located and differentiated at the adluminal region, then moved towards a central lumen of the testicular tubule and eventually became fully mature spermatozoa.

Based on the cell shapes, sizes and the chromatin organization in their nuclei, the male germ cells were categorized.

3.3.1. Spermatogonia (Sg)

Sg was a small spherical cell (approximately 6 µm in cell diameter). The cell had a fairly large amount of lightly stained cytoplasm (Figure 3A) which contained a number of rough endoplasmic reticulum. The round nucleus (diameter of 4-5 µm) contained lightly stained and loosely packed chromatin. There was only a thin strip of heterochromatin underlining the inner facet of the nuclear envelope and small blocks of heterochromatin scattered throughout the central part of the nucleus. The nucleolus was very prominent. Sg rested on the thick basal lamina that separated them from the capsular connective tissue layer.

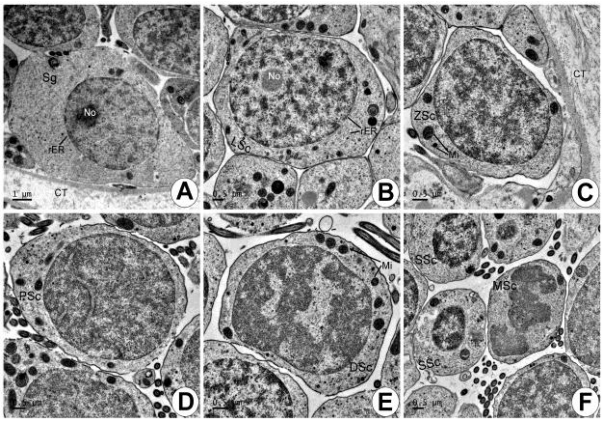

Figure 3. A: Stages of spermatogenic cells in the testicular tubules are classified based on the nuclear sizes and a pattern of chromatin condensation as spermatogonia (Sg in A), B: leptotene spermatocyte (LSc in B), C: zygotene spermatocyte (ZSc in C), D: pachytene spermatocyte (PSc in D), E: diplotene spermatocyte (DSc in E), F: metaphase spermatocyte and secondary spermatocyte (MSc and SSc in F).
CT, connective tissue trabecula; rER, rough endoplasmic reticulum; Mi, mitochondria; No, Nucleolus.

3.3.2. Spermatocytes

Spermatocytes were the most numerous cells within germinal epithelium of the testicular tubules. The increased cell size and the amount of condensing heterochromatin in the nuclei made these cells distinguishable from Sg. In each testicular tubule, most cells were primary spermatocytes appearing in various stages, while only a few secondary spermatocytes were present. The primary spermatocytes were subdivided into 6 stages: leptotene, zygotene, pachytene, diplotene, diakinetic, and metaphase spermatocytes, followed by secondary spermatocytes.

Leptotene spermatocytes (LSc)

LSc cells were slightly larger than Sg (about 7 µm in cell diameter). The round nucleus was also larger (diameter of 6 µm), thus having a higher nuclear-cytoplasmic ratio. The nucleolus was very prominent, and increased an amount of heterochromatin in small blocks were scattered throughout the nucleus (Figure 3B).

Zygotene spermatocytes (ZSc)

Major characteristics of ZSc were similar to LSc including numerous mitochondria in the cytoplasm. The heterochromatin in the nucleus (diameter of 6 µm) was increased and appeared as cords with some attached to the nuclear envelope (Figure 3C).

Pachytene spermatocytes (PSc)

PSc cells were the major population of spermatocytes in the germinal epithelium. PSc became larger (about 7-8 µm in cell diameter) with a high nuclear-cytoplasmic ratio. There were a lot of mitochondria and small vesicles in the cytoplasm (Figure 3D). The round nuclei (diameter of 6-7 µm) were completely filled with heterochromatin blocks whose fibers were attached to a thread-like scaffold. The synaptonemal complexes appeared among the heterochromatin blocks.

Diplotene spermatocytes (DSc)

DSc showed that the chromatin had condensed into large heterochromatin blocks in the nucleus and the diameters were about 7 µm (Figure 3E). As a result there were more translucent areas in the nuclei. Some large blocks of heterochromatin were attached to the nuclear envelope. Numerous mitochondria and vesicles were still present in the cytoplasm.

Diakinetic spermatocytes (DiSc) and metaphase spermatocytes (MSc)

DiSc cells were rarely seen among the developing germ cells. As the heterochromatin reached the highest condensation in the DiSc, the nuclear envelope began to break down, and MSc was later derived from DiSc. MSc appeared as irregular-shaped cells whose nuclear envelope was no longer present (Figure 3F). Very dense blocks of heterochromatin aligned together at the midline of the cells.

Secondary spermatocytes (SSc)

Following the division of MSc, SSc were derived and appeared as small round cells with nuclear diameters of 2-3 µm (Figure 3F). This cell type frequently appeared near the MSc; however, the size was a half smaller than the primary spermatocytes. Condensed blocks of heterochromatin appeared along the nuclear envelope exhibiting a clock-face pattern. Mitochondria and Golgi apparatus were clearly observed in the cytoplasm.

3.3.3. Spermatids and spermatozoa

The spermatids underwent differentiation in the adluminal region until becoming spermatozoa. Based on the condensation pattern of chromatin and the presence of cytoplasmic organelles, the differentiating spermatids were classified into 2 stages, spermatid 1 and spermatid 2. The latter transformed into mature spermatozoa.

Spermatid 1 (St1)

The St1 was a small round cell with a nuclear diameter of 2-2.5 µm and a strip of heterochromatin attached to the inner facet of the nuclear envelope (Figures 4A, 4B). The clear cytoplasm contained many mitochondria which were aggregated at one side of the cell.

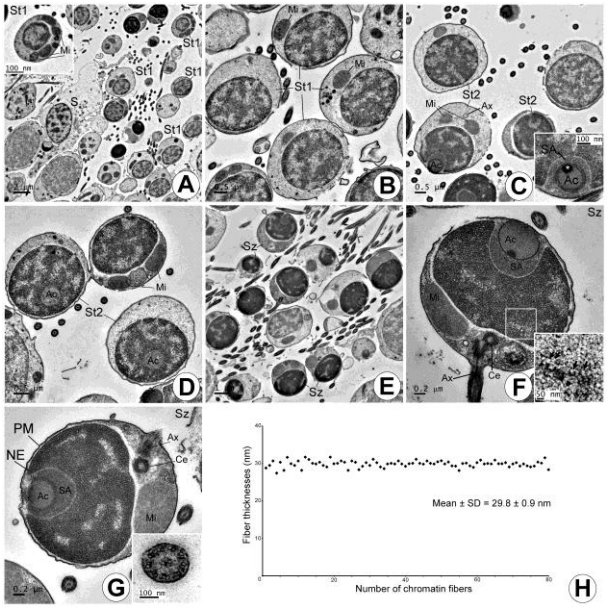

Figure 4. Differentiated spermatids during spermiogenesis mostly reside at the adluminal region of the germinal epithelium. There are two stages of spermatids, i.e., spermatid 1 (St1) (A, B), spermatid 2 (St2) (C, D), and spermatozoa (Sz) (E, F, G). Mitochondrial aggregation appears in the cytoplasm of St1 (A-inset). St2 exhibits mitochondrial enlargement at the caudal pole together with the acrosomal formation and subacrosomal materials (*) indented from the plasma membrane at the anterior pole of the cell (C, D). Higher magnification image of Sz nucleus shows that the condensed chromatin is organized from a coiling of 30-nm fibers (arrows in F-inset). Measurement of the chromatin fiber thickness (H) supports the above conclusion. Microtubular organization in the tail axoneme is shown in a cross section (G-inset).
Ac, acrosome; Ax, tail; axoneme; Ce, centrioles; Mi, mitochondria; NE, nuclear envelope; PM, plasma membrane; S, Sertoli-like cell; SA, subacrosomal material.

Spermatid 2 (St2)

The nucleus of the St2 was similar in size and shape to that of St1. However, increased chromatin condensation was the marked characteristic of St2. Heterochromatin occupied up to 40% of the nuclear cross section (Figures 4C, 4D), and it was made of the tightly packed 30-nm fibers. At the caudal pole of the St2 cytoplasm, there was an aggregation of 3-4 large mitochondria which were later rearranged into a collar-liked structure. The acrosome began to appear in the anterior pole while a large mitochondrial aggregation was located on the opposite side. A side view of St2 demonstrated that the acrosome vesicle was indented anteriorly near the plasma membrane of the anterior pole. Electron dense subacrosomal structures appeared under and around the acrosome (Figure 4C). Centrioles and microtubule axonemes were not prominent, although they were present at the caudal end of some St2 cells.

Mature spermatozoa (Sz)

The nuclear size of the Sz was about 2-3 µm and there was less cytoplasm remaining. The nuclear chromatin reached the highest degree of condensation leaving only about 30% of clear area of nucleoplasm occupied by loose chromatin fibers (Figure 4E). Within the highly condensed chromatin, small and uniform chromatin fibers, approximately 30 nm in width could still be visualized at high magnification (Figures 4F, 4G). The thickness of the chromatin fibers measured from random areas of the Sz nucleus showed an average size of 29.8 nm (Figure 4H). The acrosome vesicle was prominent and surrounded by a subacrosomal structure. A large aggregation of mitochondria was present at the neck region which also contained a pair of centrioles in the center. From the centrioles, a flagellum extended out to form the tail of the mature spermatozoa. The axonemes in the tail of the Sz appeared to be composed of a central pair and nine micro-tubule doublets which were completely covered by a thin plasma membrane.

4. Discussion and Conclusions

Most marine animals reproduce by external fertilization without copulation and insemination. Upon being stimulated by pheromones or certain chemotactic factors, the gametes from the males and females are broadcast-spawned synchronously into the sea water within the same vicinity where the fertilization of the eggs takes place quickly. For this to be successful, the gonads usually have a fairly simple structural organization of a simple branching tubular gland in which multiple tubules converge into a large and relatively short common duct that opens immediately to the exterior at the head region. Compared to the animals which adopted the internal fertilization, as practiced by higher invertebrates and vertebrates, the numbers of gametes being released by the animals with external fertilization are usually many times higher to ensure the reproductive success. This is enabled by rapid proliferation and differentiation of germ cells within the wall or germinal epithelium of the gonadal tubules. The testis of the sea cucumbers exhibit this simple form of structural organization whereby multiple tubules converge together to form a short spermatic duct that open at the gonopore close to the mouth (Demeuldre & Eeckhaut, 2012; Ramofafia, Battaglene, Bell, & Byrne, 2000). In *H. scabra*, each testicular tubule branches twice to increase the surface area of the germinal epithelium for sperm production so that the mature sperm can be massively produced and released through the gonopore upon a contraction of the testicular tubules. Although most of the testicular tubules are believed to be formed at the same time, each individual tubule seems to contain a group of spermatogenic cells that proliferate and differentiate independently (Hamel, Conand, Pawson, & Mercier, 2001; Ramofafia *et al.*, 2003; Rasolofonirina *et al.*, 2005). As a result, some testicular tubules may not have mature sperm ready while several tubules nearby are about to release the mature sperm.

Thick tubular wall of *H. scabra* testis provides sturdy support for the germinal epithelium as well as helping to maintain appropriate internal milieu for the proliferating and differentiating germ cells. The ultrastructural features of the two types of the outer epithelium lining the surface of the testicular tubule are remarkable. The vacuolated cells may have a nutritive role as they their vacuoles, though pale-stained, were still filled with flocculent materials which could contain some nutritive substances taken up from the coelomic fluid. The second type of cells contains dense granules and some lipid droplets. Their role could be in lipid storage and endocrine function. However, their actual roles remain to be investigated. The thick connective tissue layer in the tubular wall and the inward extensions into the trabeculae are composed of high contents of collagenous and elastic fibers which serve as strong support for the proliferative germ cells. However, they allow flexibility as the tubules contract by the action of the adjacent muscular layer. The longitudinal layer of muscles around the testicular wall is believed to act in a rhythmic contraction during the gamete release as reported in other echinoderms (Okada & Iwata, 1985; Okada, Iwata, & Yanagihara, 1984).

Our study has demonstrated that the stages of proliferating and differentiating germ cells in the *H. scabra* testis resemble those that occur in several other echinoderms (Atwood & Chia, 1974; Floriana, 1995;Sousa & Azevedo, 1988) and vertebrates including mammals (Cheng & Mruk, 2010; Clermont, 1972; Guraya, 1987). However, only a single pattern of cellular association appears in one cross section of the testicular tubule. This pattern is composed of the sequential proliferation and differentiation starting from the Sg, which undergo mitotic division to yield the LSc whose nucleus enters the prophase of the first meiotic division and give rises to subsequent stages of primary spermatocytes that include the ZSc, PSc, DSc, DiSc and MSc. These cells are characterized by progressive thickening of the chromatin from the thread-like fibers in the LSc to thickened cords, which are paired up by the synaptonemal complexes in the ZSc and PSc. The cords become the thickest in the PSc. Then, the chromatin cords transform to chromosomes in the DSc and DiSc and eventually the complete chromosomes that become aligned at the equatorial region in the MSc stage. MSc later divides into two SSc, a transient stage that gives rise to two spermatids.

During spermiogenesis, spermatids undergo the process of cellular differentiation and morphological trans-formation into mature spermatozoa. Four basic phases of spermatid transformation have been classically identified in most animals including the Golgi phase, the cap phase, the

acrosomal phase, and the maturation phase, respectively (Clermont, 1972; Holstein, 1976). The key structures derived from these events are the acrosome, the sperm flagellum, the cytoplasm shedding, and the condensation of the chromatin together with the nuclear shaping (De Kretser, 1969; Fawcett, Anderson, & Phillips, 1971). The former also controls the shape of the sperm head (nucleus) which has species-specific characteristics. Echinoderm sperm exhibit a variety of shapes such as the round-headed sperm in *Thyone briareus* (Inoué & Tilney, 1982), and the cylindrical-headed sperm in *Cucumaria lubrica* (Atwood & Chia, 1974). The holothurian sperm are considered to be a relatively primitive form that are adapted for external fertilization as they assume a small, round-to-conical shape (Chia, Atwood, & Crawford, 1975). The spherical head of *H. scabra* sperm allows them to readily swim away once they are detached from the germinal epithelium. Our TEM images of *H. scabra* have demonstrated the presence of dense subacrosomal material in the subacrosomal space during late spermatids and spermatozoa. This electron dense material is thought to be a massive accumulation of actin. Following sperm activation after contact with eggs, actin is polymerized into bundles that are able to push the acrosome forward, leading to lysis of the egg coat by the released acrosomal enzymes that pave the way for sperm-egg membrane fusion. After being activated by the egg's jelly coat, sperm of sea urchins and sea stars also exhibit actin polymerization in the subacrosomal space that forms a long extending filamentous structure that pushes the acrosome forwards (Marks, Biermann, Eanes, & Kryvi, 2008; Nakachi, Moriyama, Hoshi, & Matsumoto, 2006). However, in the sea cucumber it is still unclear how sperm activation is triggered and by which mechanism it binds and penetrates through the egg coatings.

In various animal species, the condensation of sperm chromatin is instrumental in the packaging and delivery of genetic materials without being damaged until the fusion of the male and female pronuclei occurs. Mammalian sperm need to travel a relatively long distance in the female reproductive tract until they meet up with the eggs, hence the sperm histones are replaced with protamines, the arginine-rich basic nuclear protein that helps to pack the chromatin fibers into the most condensed state (Brewer, Corzett, & Balhorn, 1999; Manfredi Romanini *et al.*, 1986; Ward, 2010). During a fusion of the male and the female pronuclei, the highly condensed sperm chromatin becomes decondensed, and protamines are subsequently replaced by oocyte-derived histones (Van der Heijden *et al.*, 2008; Van Meel & Pearson, 1979). According to Ausio *et al.*, (1999), the sperm of most vertebrates including rodents, humans, and fish that reproduce by internal fertilization are classified as the protamine type. In animals that reproduce by external fertilization the sperm and eggs are released simultaneously within the same vicinity and the sperm do not need to swim far before meeting up with the eggs. Therefore, they either use less basic proteins such as the protamine-like proteins for DNA packaging as in shellfish sperm or the sperm-specific histones with some variances as in frog sperm, or even only somatic histones as in prawn and crab sperm (Itoh, Ausio, & Katagiri, 1997; Lewis & Ausio, 2002). Our data demonstrated that the condensed chromatin of *H. scabra* sperm is organized from the not-so-tightly-packing somatic type 30-nm fibers (Figure 5). This pattern of chromatin condensation is similar to that observed in frog sperm (Manochantr, Sretarugsa, Chavadej, & Sobhon, 2005), abalone, and bivalve sperm (Bernard & Hodgson, 1985; Casas & Subirana, 1994; Kang, Chung, Kim, Chung, & Lee, 2012; Suphamungmee *et al.*, 2005), which use some histone variance proteins for chromatin packaging. Hence, we speculate that this rather loosely packed chromatin of the sea cucumber sperm may be mediated by some sperm-specific histones. This was supported by the study in the sea urchin sperm, another closely related echinoderm species, where histone-like proteins are used for packaging chromatin (Poccia, Simpson, & Green, 1987; Romano, 1992). However, the true identity of histone variance that is used in the packaging of the sea cucumber sperm chromatin is still not conclusive and it is the subject of our current study.

Other than spermatogenic cells, the Sertoli-like cells are also present among the germ cells of *H. scabra*. This cell type has a relatively large nucleus with dense patches of heterochromatin, and its branching cytoplasm contains multiple lysosomes and autophagosomes. Cells with similar characteristics identified in the sea urchin testis are called nutritive phagocytic (NP) cells (Reunov *et al*., 2004). This specialized cell contains abundant autophagic vesicles which are likely involved in macroautophagy and microautophagy processes. The NP cell of the sea urchin has been suggested to function in the storage of nutrients and in the degradation of the residual cytoplasm of the terminal stage of spermatogenic cells via phagocytosis (Kalachev & Yurchenko, 2016). In the sea cucumber testis, the Sertoli-like cells reside in both the basal and the adluminal compartments of the germinal epithelium. Therefore, we believe that they are involved in supplying nutrients to spermatogenic cells and also phagocytose the sperm's residual cytoplasm in their final stage of differentiation.

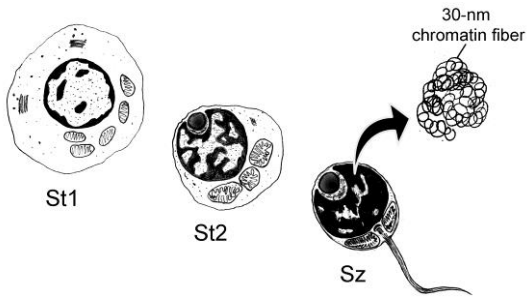

Figure 5. Schematic drawing summarizes the features of the two stages of spermatids and spermatozoa during spermiogenesis of *H. scabra* which consist of spermatid 1 (St1), spermatid 2 (St2), and spermatozoa (Sz). A magnified picture on the upper right hand illustrates a proposed model of chromatin condensation in the nuclei of sperm from the coiling and compaction of 30-nm fibers.

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References

Atwood, D. G., & Chia, F. S. (1974). Fine structure of an unusual spermatozoon of a brooding sea cucumber *Cucumaria lubrica*. *Canadian Journal of Zoology, 52*, 519-523.

Bernard, R. T. F., & Hodgson, A. N. (1985). The fine structure of the sperm and spermatid differentiation in the brown mussel *Perna perna*. *Journal of African Zoology, 20*, 5-9.

Brewer, L. R., Corzett, M., & Balhorn, R. (1999). Protamine-induced condensation and decondensation of the same DNA molecule. *Science, 286*, 120-123.

Casas, M. T., & Subirana, J. A. (1994). Chromatin condensation and acrosome development during spermiogenesis of *Ensis ensis* (Mollusca, Bivalvia). *Molecular Reproduction and Development, 37*, 223-228.

Cheng, C. Y., & Mruk, D. D. (2010). The biology of spermatogenesis: The past, present and future. Philosophical Transactions of the Royal Society of London. Series B, *Biological Sciences, 365*, 1459-1463.

Chia, F. S., Atwood, D. G., & Crawford, B. (1975). Comparative morphology of echinoderm sperm and possible phylogenetic implications. *American Zoologist, 15*, 533-565.

Clermont, Y. (1972). Kinetics of spermatogenesis in mammals: Seminiferous epithelium cycle and spermatogonial renewal. *Physiology Review, 52*, 198-236.

Conand, C. (1998). Holothurians. In K. E. Carpenter & V. H. Niem (Eds.), *FAO species identification guide for fishery purposes. The living marine resources of the Western Central Pacific.Volume 2: Cephalopods, crustaceans, holothurians and sharks* (pp. 1157-1150). Rome, Italy: Food and Agriculture Organization of the United Nations.

De Kretser, D. M. (1969). Ultrastructural features of human spermiogenesis. *Zeitschrift für Zellforschung und mikroskopische Anatomie, 98*, 477-505.

Demeuldre, M., & Eeckhaut, I. (2012). Gonad development in the sea cucumber *Holothuria scabra* Jaeger, 1833. *SPC Beche-de-mer Information Bulletin, 2*, 15-23.

Fawcett, D. W., Anderson, W. A., & Phillips, D. M. (1971). Morphogenetic factors influencing the shape of the sperm head. *Developmental Biology, 26*, 220-251.

Floriana, R. (1995). Sperm-egg interactions during fertilization in invertebrates. *Bollettino di Zoologia, 624*, 323-334.

Guraya, S. S. (1987). *Biology of Spermatogenesis and Spermatozoa in Mammals.* London, England: Springer-Verlag.

Hamel, J. F., Conand, C., Pawson, D. L., & Mercier, A. (2001). The sea cucumber *Holothuria scabra* (Holothuroidea: Echinodermata): its biology and exploittation as Beche-de-mer. *Advance Marine Biology, 41*, 129-223.

Holstein, A. F. (1976). Ultrastructural observations on the differentiation of spermatids in man. *Andrologia, 8*, 157-165.

Inoué, S., & Tilney, L. G. (1982). The acrosomal reaction of Thyone sperm. I. Changes in the sperm head visualized by high resolution video microscopy. *Journal of Cell Biology, 93*, 812-819.

Itoh, T., Ausio, J., & Katagiri, C. (1997). Histone H1 variants as sperm-specific nuclear proteins of *Rana catesbeiana*, and their role in maintaining a unique condensed state of sperm chromatin. *Molecular Reproduction and Development, 47*, 181-190.

Kalachev, A. V., & Yurchenko, O. V. (2016). Micro-autophagy in nutritive phagocytes of sea urchins. *Protoplasma, 254*(1), 609-614. doi: 10.1007/s00709-016-0963-1

Kang, H. W., Chung, E. Y., Kim, J. H., Chung, J. S., & Lee, K. Y. (2012). Germ cell differentiations during spermatogenesis and taxonomic values of mature sperm morphology of Atrina (Servatrina) pectinata (Bivalvia, Pteriomorphia, Pinnidae). *Developmental Reproduction, 16*, 19-29.

Lewis, J. D., & Ausio, J. (2002). Protamine-like proteins: Evidence for a novel chromatin structure. *Biochemistry and Cell Biology, 80*, 353-361.

Manfredi Romanini, M. G., Biggioggera, M., Formenti, D., Fraschini, A., Garagna, S., Pellicciari, C., & Redi, C. A. (1986). Sperm chromatin maturation in the mouse. A cytochemical approach. *Histochemistry, 84*, 484-491.

Manochantr, S., Sretarugsa, P., Chavadej, J., & Sobhon, P. (2005). Chromatin organization and basic nuclear proteins in the male germ cells of *Rana tigerina*. *Molecular Reproduction and Development, 70*, 184-197.

Marks, J. A., Biermann, C. H., Eanes, W. F., & Kryvi, H. (2008). Sperm polymorphism within the sea urchin *Strongylocentrotus droebacmhiensis*: Divergence between Pacific and Atlantic Oceans. *Biological Bulletin, 215*, 115-125.

Nakachi, M., Moriyama, H., Hoshi, M., & Matsumoto, M. (2006). Acrosome reaction is subfamily specific in sea star fertilization. *Developmental Biology, 298*, 597-604.

Okada, Y., Iwata, K. S., & Yanagihara, M. (1984). Synchronized rhythmic contractions among five gonadal lobes in the shedding sea urchin: Coordinative function of the aboral nerve ring. *Biological Bulletin, 166*, 228-236.

Okada, Y., & Iwata, K. S. (1985). A substance inhibiting rhythmic contraction of the gonad in the shedding sea urchin. *Zoological Science, 2*, 805-808.

Poccia, D. (1986). Remodeling of nucleoproteins during gametogenesis, fertilization, and early development. *International Review of Cytology, 105*, 1-65.

Poccia, D. L., Simpson, M. V., & Green, G. R. (1987). Transitions in histone variants during sea urchin spermatogenesis. *Developmental Biology, 121*, 445-453.

Ramofafia, C., Battaglene, S. C., Bell, J. D., & Byrne, M. (2000). Reproductive biology of the commercial sea cucumber *Holothuria fuscogilva* in the Solomon Islands. *Marine Biology, 136*, 1045-1056.

Ramofafia, C., Byrne, M., & Battaglene, S. C. (2003). Reproduction of the commercial sea cucumber *Holothuria scabra* (Echinodermata: Holothuroidea) in the Solomon Islands. *Marine Biology, 142*, 281-288.

Rasolofonirina, R., Vaïtilingon, D., Eeckhaut, I., & Jangoux, M. (2005). Reproductive cycle of edible echinoderms from South-Western Indian Ocean: II. The sandfish *Holothuria scabra* (Jaëger, 1833). *Western Indian Ocean Journal of Marine Science, 4*, 61-75.

Romano, G. (1992). Histone variants during sea urchin development. *Cell Biology International Reports, 16*, 197-206.

Sewell, M. A., Tyler, P. A., Young, C. M., & Conand, C. (1997). Ovarian development in the class Holothuroidea: A reassessment of the tubule recruitment model. *Biological Bulletin, 192*, 17-26.

Sousa, M., & Azevedo, C. (1988). Fine structural study of the acrosome formation in the starfish *Marthasterias glacialis* (Echinodermata, Asteroidea). *Tissue and Cell, 20*, 621-628.

Suphamungmee, W., Apisawetakan, S., Weerachatyanukul, W., Wanichanon, C., Sretarugsa, P., Poomtong, T., & Sobhon, P. (2005). Basic nuclear protein pattern and chromatin condensation in the male germ cells of a tropical abalone, *Haliotis asinina*. *Molecular Reproduction and Development, 70*, 211-221.

Summers, R. G., Hylander, B. L., Colwin, L. H., & Colwin, A. L. (1975). The functional anatomy of the echinoderm spermatozoon and its interaction with the egg at fertilization. *American Zoology, 15*, 523-551.

Van Meel, F. C., & Pearson, P. L. (1979). Replacement of protamine by F1 histone during reactivation of fused human sperm nuclei. *Histochemistry, 6*, 329-339.

Van der Heijden, G. W., Ramos, L., Baart, E. B., Van den Berg, I. M., Derijck, A. A., Van der Vlag, J., . . . De Boer, P. (2008). Sperm-derived histones contribute to zygotic chromatin in humans. *BMC Developmental Biology, 8*, 34.

Walker, C. W., Unuma, T., & Lesser, M. P. (2013). Sea urchin gametogenesis - structural, functional and molecular/genomic biology. In J. M. Lawrence (Ed.), *Developments in Aquaculture and Fisheries Science* (pp. 25-43). San Diego, CA: Academic Press.

Ward, W. S. (2010). Function of sperm chromatin structural elements in fertilization and development. *Molecular Human Reproduction, 16*, 30-36.