Testicular structure and spermatogenesis of short mackerel, *Rastrelliger brachysoma* (Bleeker, 1851) in Upper Gulf of Thailand

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Abstract. Testicular structure and spermatogenesis of short mackerel, *Rastrelliger brachysoma* (Bleeker, 1851) (Teleostei: Scombridae) was first investigated. The testicular parenchyma was a lobular organ, which was classified as an unrestricted spermatogonial type. The classification of spermatogenetic stage could be classified into six stages based on the pattern of chromatin condensation and other characterizations at the light microscopic level. These six stages included the primary and secondary spermatogonium, primary and secondary spermatocyte, spermatid and spermatozoon. The spermatogenesis could also be classified into another four stages based on the nuclear and cytoplasmic characterizations at the ultrastructural level. Spermatogonium was the early germ cell. It underwent a series of mitotic division to reach the primary spermatocyte. Secondary spermatocyte was shown as the heterochromatin surrounding the nuclear membrane, which was rarely seen within seminiferous lobules. Stages during the spermatids differentiation comprised of the early, intermediate and late stages which are under the degree/change of chromatin condensation. Finally, the spermatozoon was revealed as the aquasperm primitive type. It was composed of an oval head without an acrosome, a short mid-piece consisting of two basal bodies (proximal and distal centrioles) and a long flagella tail without lateral fins. The axonemes of classical form with 9+2 microtubules were presented in the flagellum.

Keywords: histology, *Rastrelliger brachysoma*, testis, Thailand

INTRODUCTION

Investigations in the testicular structure and spermatogenesis under light microscopic level, have been observed in several orders of teleost i.e., perciform, cypriniform, atheriniform and beloniform (Parenti and Grier, 2004; Dietrich and Krieger, 2009). Similarly, ultrastructural studies of the spermatogenic stages have also been investigated in teleost fish in Families: Anguillidae (Todd, 1976), Mugilidae (Brusle, 1981), Poeciliidae (Billard, 1984), Sparidae (Gwo *et al.*, 1993), Characidae (Romagosa *et al.*, 1999), Scombridae (Abascal *et al.*, 2002), Synbranchidae (Lo Nostro *et al.*, 2003) and Gobiidae (Chung,

2008). The sperm morphology was particularly investigated because it varied in its shape when compared to other vertebrate groups (Mattei, 1991; Jamieson and Leung, 2011). The interest in sperm morphology involved many aspects in fish biology such as reproductive biology, physiology, phylogenetic analysis and breeding (Jamieson and Leung, 1991; Mattei, 1991). Similarly in previous observation, the spermatozoa morphology in teleost fish have been used as a powerful tool in taxonomic and phylogenetic classification (Jamieson and Leung, 1991; Mattei, 1991). Not only a useful tool for the systematical analysis, but

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also for the diversity of habitats and reproductive modes of the fishes. Surprisingly, spermatozoa morphology can be mainly classified into two types. A simple first type is the aquasperm spermatozoon, characterized by a round head with a short neck region (mid-piece), and few mitochondrias, whereas in the second type, the introsperm spermatozoon has the elongated head and other derived features including absence of the mid-piece and nuclear rotation (Jamieson and Grier, 1993; Jamieson, 2011; Mattei, 1991). Differences between these two types reflect the mode of fertilization in fishes (Jamieson and Grier, 1993; Jamieson, 2011). Among fishes, several scombrids such as *Scomberomorus tritor* (Mattei, 1991), *Thunnus thynnus* and *Euthynnus alletteratus* (Abascal *et al.*, 2002), *Scomber australasicus* (Hara and Okiyama, 1998), *S. japonicas* (Mattei, 1991; Hara and Okiyama, 1998) and *Cybbium tritor* (Mattei, 1991) have especially observed due to their economical important value and investigated in male germ cells based on fine structure.

Unfortunately, no information has been done on the structure using light and electron microscopes of the spermatogenesis in the short mackerel *Rastrelliger brachysoma*. It is considered as the only marine species that is successfully managed in Thailand. However, due to recent reducing in sign of age of maturity, the Thai government is operating an aquaculture program for it. Therefore, it is the best candidate for further research as it is the promising prospect for aquaculture in Thailand. Hence, in this study, the testicular structure and spermatogenic stages of the *R. brachysoma* living in the Upper Gulf of Thailand were clarified using light and transmission electron microscopes.

MATERIALS AND METHODS

Animals and sampling. Adult *R. brachysoma* (15 to 18 cm standard length) were used in this study. Fish were collected by bamboo strake trap from Samut Songkram Province on the Upper Gulf of Thailand (13°16'18.4"N, 100°02'13.4"E), during non-breeding season (October to December 2013, *n* = 20) and breeding season (January to February 2014, *n* = 20). These seasons were classified by the testicular development from our

previous reports (Senarat *et al.*, 2016; Senarat *et al.*, unpublished data). All fish were euthanized by rapid cooling shock (Wilson *et al.*, 2009). The experimental protocol was approved by the Animal Care and Use Committee of Faculty of Science in accordance with the guide for the care and use of laboratory animal prepared by Chulalongkorn University (Protocol Review No. 1423003).

Light microscopic observation. The testis tissues were immediately fixed in Davidson's fixative for 48 hours at room temperature and processed using standard histological. The testicular paraffin block was cut at 5 µm thickness and stained with Harris' hematoxylin and eosin (H&E) (Bancroft and Gamble, 2002). For histochemical technique, the basement membrane, reticular fiber, protein, carbohydrate and lipid contents of testis tissue were specific stained with Masson's trichrome (MT), periodic acid-schiff (PAS), alcian blue pH 2.5 (AB), germ positive (GP) and reticulin (RT) staining methods (modified from Puchtler and Waldrop, 1978; Vidal, 1988; Bancroft and Gamble, 2002). Additionally, frozen tissues were cut at 10 µm thickness by freezing microtome and stained with oil red O (ORO) (modified from Culling, 1963).

Ultrastructural observation. Small pieces of testicular tissue (approximately 1 mm³ in size) were cut and rapidly prefixed in 2.5% glutaraldehyde (phosphate buffer pH 7.3) for 24 hours at 4°C and postfixed in 1% osmium tetroxide. Testicular tissues were dehydrated in serial graded ethanol throughout embedding in epoxy resin (Epon 812). The semithin tissues were cut at 500 nm thickness, then stained with toluidine blue. Ultrathin sections were cut at 90 nm thickness, then stained with uranyl acetate and lead citrate. The sections were observed with a JEM-2100 (200kV) transmission electron microscope (TEM, JEOL, Japan).

RESULTS

Histology of testicular tissue. Asymmetrical macroanatomy of *R. brachysoma* testes had paired organs and heart shape in cross section (Figures

1A, 1B). They are situated in the dorsal part of the abdominal cavity, under the kidney. The cross sections revealed that the parenchyma of the testicular tissue was the anastomosing lobular type, which was surrounded by tunica albuginea. Its tunica albuginea was covered by mesothelium similar to the ovary (Figure 1A). The projection of tunica albuginea extended into the testicular parenchyma, forming lobules in the dorsal region, which was divided into two compartments (Figures 1A, 1D): (i) the interstitial compartment which contained the blood/lymphatic vessels, perilobular myoid cells and steroidogenic Leydig cells (Figures 1R, 1S). Perilobular myoid cells were elongated in shape. It was also showed the weakly acidophilic cells with surrounding the lobular. Steroidogenic Leydig cells had a large polygonal shape and were usually represented as small groups near blood capillaries in the interstitial compartment. Their nuclei were basophilic containing one nucleolus. (ii) The germinal compartment contained the numerous seminiferous lobules. The testis of *R. brachysoma* was classified as unrestricted spermatogonial type because the spermatogonia occurred and were distributed along the entire length of the germinal compartment of the lobules (Figure 1E). Each seminiferous lobule composed of spermatogenic and somatic sertoli cells. The somatic sertoli cells had triangular nuclei with large, prominent nucleoli and slightly acidophilic cytoplasm. They were usually presented in low numbers and located adjacent to lobular septa. Each stage of spermatogenesis was synchronously developed, called spermatocyst.

Based on the structural features, such as cell size, shape, nuclear characteristics, chromatin condensation, amount of cytoplasm and staining properties, the spermatogenic process of *R. brachysoma* can be classified as follows:

Primary spermatogonium was the largest among the spermatogenic cells with diameter about 10-12 μm . It was generally located as single cell near the seminiferous epithelial compartment. The center of the oval nucleus contained a very prominent single nucleolus with weakly basophilic nucleoplasm. The granular cytoplasm contained moderate amounts (Figure 1F).

Secondary spermatogonium derived from the primary spermatogonium by mitosis division. This stage was similar in character to the previous

stage, but the nucleus had stronger basophilia than the primary spermatogonium due to the increase of heterochromatin condensation along the nuclear membrane. Each group (generally more than 4 cells) with 12-15 μm in diameter was commonly found in cysts, called spermatocysts (Figures 1C, 1G).

Primary spermatocyte was formed when the secondary spermatogonia enters meiosis. Its size (7-8 μm) was smaller than secondary spermatogonia and still occurred inside spermatocyst. The spherical or oval nucleus of secondary spermatocyte had a granule and stained as a basophilic cell with no prominent nucleolus. It exhibited a moderate amount of distinct acidophilic cytoplasm (Figure 1H).

Secondary spermatocyte, developed from primary spermatocyte under the first meiotic division. It was relatively smaller in size, about 6-7 μm than that of primary spermatocytes. It contributed to the largest spermatocyst (35-40 μm). The distinguishing feature of this stage was a change in shape and color of the nucleus; round nucleus increasingly condensed near the nuclear membrane without nucleolus (5 μm) as basophilic chromatin, which did not contain. The basophilic staining of the second spermatocyte decreased as it depended (Figure 1I). However, it was rarely observed throughout the testis.

Spermatid derived from secondary spermatocyte after the second meiotic division. It was still contained inside spermatocyst. The oval nucleus was small with intensive basophilic. Rims of eosinophilic cytoplasm were shown, due to loss of its cytoplasm during spermiogenesis (Figures 1C, 1J).

Spermatozoon was the smallest spermatogenic cell. It was found in the lumen because of the spermatocyst capsule breaking out and then being released into vasa deferentia. It can be morphologically distinguished into 2 regions: head and tail. The ovoid head of the spermatozoon was responsible for its chromatins becoming completely condensed and was represented as strongly basophilic. The tail was strongly acidophilic with rarely apparent unflagella in histological sections (Figures 1C, 1K). The spermatozoa within lumen which were released during spermiation into the ventral region were called the anastomosing zone before

opening and continued with the vasa efferent, respectively.

Histochemistry of testicular tissue

Protein and carbohydrate. Histochemistry of the testicular tissue detected by AB (Figures 2A, 2B) as slightly positive as well as MT (Figures 2E,

2F) and PAS (Figures 2G, 2H) had strong positives for tunica albuginea and interstitial connective tissue, while spermatogonia and primary spermatocytes were slightly positive with PAS reaction. Both Leydig cells and somatic sertoli cells were moderately stained with the same reaction.

Figure 1. Light photomicrograph and schematic summary of testicular structure (A-E) and spermatogenesis (F-S). Note: blood vessel (Bv), germinal compartment (Gnc), head of spermatozoa (H), interstitial compartment (Itc), Leydig cells (Lec), myoid cells (Myc), nucleus (N), primary spermatocyte (Psc), primary spermatogonia (Psg), seminiferous lobule (SL), spermatocyst (Spt), secondary spermatocyte (Ssc), secondary spermatogonia (Ssg), spermatids (St), sertoli cell (Stc), spermatozoa (Sz), tail of spermatozoa (T), tunica albuginea (Ta), testis (Te), vasa efferentia (Ve). Scale bar A, C, D = 100 μ m.

Figure 2. Light photomicrograph of testicular structure and spermatogenesis. (A-B) based on alcian blue pH 2.5 (AB), (C-D) based on oil red O (ORO), (E-I) based on Masson's trichrome (MT), and (H-J) based on periodic acid -schiff (PAS). Note: blood vessel (Bv), Leydig cell (Lc), primary spermatocyte (Psc), primary spermatogonia (Psg), seminiferous lobule (SL), secondary spermatocyte (Ssc), secondary spermatogonia (Ssg), spermatids (St), spermatozoa (Sz), tunica albuginea (Ta). Scale bar A, D, E, F, G, H = 100 μ m; B, C, I, J = 50 μ m.

Lipid. Overall testicular tissues were unstained with ORO, indicating the absence of lipids (Figures 6C, 6D).

Fiber composition. Reticulin reaction from longitudinal section revealed that the several blind ends of the lobule testis were established and protruded by tunica albuginea. Moreover, cross sections from the testis parenchyma also showed similar pattern. Basement membrane was obviously observed between germinal and interstitial compartments, respectively (Figures 3A, 3B).

Figure 3. Light photomicrograph of testicular structure and spermatogenesis (A-B). Note: basement membrane (Bm), primary spermatocyte (Psc), primary spermatogonia (Psg), seminiferous lobule (SL), secondary spermatogonia (Ssg), spermatids (St), spermatozoa (Sz). Scale bar A, B = 50 μ m.

Ultrastructure of testicular tissue. Based on semithin section, normal testis of the adult *R. brachysoma* showed that the lobular lumen in germinal compartment contained various stages of spermatogenic cells during the reproductive season. Ultrastructure evidences e.g., cytoplasmic morphology and chromatin condensation of the nucleus were observed with TEM. Spermatogenesis in this species could be classified into 4 successive stages as follows:

Spermatogonium (approximately 10-12 μ m) was the largest male germ cells and occurred in small groups at the periphery of the seminiferous lobule (Figure 4A). TEM revealed that the spermatogonium was either slightly spherical or oval in shape. Its nucleus was large and prominent oval in shape, which was occupied by the presence of fine granular chromatin throughout the nucleoplasm. At this stage, a few cytoplasmic

organelles such as several globular mitochondria were present (Figure 4B).

Primary spermatocyte originated mitotically from the spermatogonium. During the beginning of meiotic prophase, it was smaller than spermatogonia with approximately 8-9 μ m (Figure 4C). Under TEM, the nucleus of primary spermatocyte was the central oval-shape and the nucleolus was not visible. At this stage, the nucleus also contained densely packed chromatin as a heterochromatic nucleus (compared to spermatogonia). During pachytene stage under the meiotic division I, there was an increasing condensation of heterochromatin among several synaptonemal complexes within the nucleus. The cytoplasm contained the endoplasmic reticulum, the electron dense particles, nuage and several mitochondria that became irregular in shape and electron dense matrix (Figures 4D, 4E). Intercellular bridge was observed at this stage.

Secondary spermatocyte was produced from the first mitotic division of primary spermatocytes. They could be identified at the structural level as having larger cysts when compared to the previous stages, but they were rarely seen with seminiferous lobules. A characteristic of the cell and nucleus were reduction in sizes (6-7 μ m) (Figure 4F). Ultrastructurally, the identifying characteristic of spherical nucleus was the appearance of the heterochromatin pattern around the nuclear membrane. The cytoplasm was reduced and still contained a few organelles. Intercellular bridge also frequently connected adjacent secondary spermatocytes (Figure 4F). This stage gave rise to spermatids under meiotic division II.

Spermatid was seen under chromatin condensation and cytoplasmic organelles changes during spermatid differentiation, called as spermiogenesis (Lahnsteiner and Patzner, 1990; Gwo *et al.*, 1993). It could be classified into three morphological stages based on the modified criterion used in other families (Lahnsteiner and Patzner, 1990; Manni and Rasotto, 1997; Quagio-Grassiotto and Oliveira, 2008). The early spermatid of spermiogenesis (approximately 4.0-4.5 μ m) had a large and round nucleus shape (Figure 5A). Under TEM, remodelling of the chromatin condensation appeared, resulting in a granular-chromatin mass (Figure 5B). At this time, cytoplasmic organelles, such as spherical-

mitochondria and centriole initially accumulated at the posterior regions (data not shown). The intermediate spermatid showed that the chromatin condensation of nucleus transformed into a more compact form, when compared with the previous stage. Also at this stage, the sphere-shaped mitochondria was located near the posterior region of the nucleus (Figures 5C, 5D). The late spermatid, the proximal centriole was located within the nuclear fossa. Their flagellum axoneme which was arranged by mitochondria was located in the mid-piece. They were also separated from the flagellum by the cytoplasmic canal (Figures 5E, 5F). The chromatin completely condensed throughout the nucleus due to increase of dense and thick chromatin granules. The cytoplasm started to degenerate. The residual bodies in a various sizes and shapes were also found in the cytoplasm (Figure 5G).

Figure 4. Photomicrographs and transmission electron micrographs of spermatogenesis showing different types of male germ cells. (A-B) spermatogonia (Sg), (C-E) primary spermatocyte (Psc), (F-G) secondary spermatocyte (Ssc). Note: electron dense particle (Ep), endoplasmic

reticulum (ER), heterochromatin (H), patch of heterochromatin (Hc), intercellular bridge (Ib), mitochondria (m), nuage (n), somatic sertoli cell (Sec), syntoplasmic complex (Sn). Scale bar A = 25 μ m; C and F = 20 μ m.

Figure 5. Photomicrograph and transmission electron micrographs showing different stages of spermatids. (A) semithin section of various spermatids, (B-C) early spermatids (Est), (D-F) intermediate spermatids (Ist), (G) late spermatids (Lst). Note: chromatin granule (Cg), flagella (F), mitochondria (m), residual bodies (Rb). Scale bar A = 25 μ m.

Spermatozoon, the mature spermatozoon had three distinct regions: a head, mid-piece and tail region (flagellum). Based on our observation, the spermatozoon of *R. brachysoma* had characteristics of those belonging to the primitive type, named aquasperm primitive type. If it is considered based on sperm characterization by van der Straten *et al.* (2006), it will be classified as Type II. The asymmetrical head region appeared as ovoid nucleus, about 2-3 μ m in length and 3-4 μ m in width, due to extreme granular chromatin

condensation with an acrosome-less head in the anterior region. The short mid-piece was cylindrical of approximately 0.5 μm . Inserted in the mid-piece, the centriolar complex was present outside the shallow nuclear fossa in bell-shape of ultrathin sections, which was composed of the (i) proximal centriole near the nucleus envelope, and (ii) distal centriole giving rise to the sperm flagellum. This region completely composed of several spherical mitochondria (approximately 0.5-0.7 μm) which were irregular cristae and moderately electron-dense. The sperm tail

(flagellum), without lateral fins of this species, was estimated about 15-18 μm . It mainly consisted of nine triplet's doubles of peripheral microtubules and axonemal doublet of the microtubules. Thus, each axonemal pattern of the flagella has a typical 9+2 arrangement of microtubules as typically presented in primitive species. A cross section of mid-piece was interconnected by Y-shaped bridge with enclosure by a flagellum plasma membrane and the flagella exhibited no lateral fins (Figures 6B, 6G, 7A, 7G).

Figure 6. Transmission electron micrographs showing late spermatids (Lst) and spermatozoa (Sz). (A) late spermatids, (B-G) the characterization of spermatozoa. Note: axoneme (ax), cytoplasmic canal (Cc), distal centriole (Dc), flagella (F), head (H), mitochondria (m), mid-piece (Mp), nucleus (N), nuclear fossa (Nf), proximal centriole (Pc), tail (T).

Figure 7. Schematic of several sections showing the characterization of spermatozoa. Note: distal centriole (Dc), head (H), mitochondria (M), mid-piece (Mp), nucleus (N), proximal centriole (Pc), tail (T).

During the reproductive season, somatic sertoli cell and perilobular myoid cell were also observed. As to detail, the localization of somatic sertoli cell was exclusively found in the periphery of the lobule and around the spermatogonia. Sertoli cell occurred within the germinal compartment of the spermatogonia. Based on a light microscope study, they were difficult to identify (Figure 5A) but they had a triangular or oval shape with a nucleus which is regular oval with scattering dense heterochromatin under TEM (Figure 8B). Also, the cytoplasm specially contained a few glycogen and several residual bodies which could be classified into two types: laminar bodies and lipofuscin pigments (Figure 8C). Therefore, this characterization underwent active phagocytosis. Several mitochondria and rough endoplasmic reticulum were observed. Each cell was joined by desmosome and tight junctions. Perilobular myoid cells were present near somatic sertoli cell (Figure 8A). Ultrastructurally, they had irregular shape, containing oval or irregular nucleus. The cytoplasm of this cell was mainly filled with high concentration of contractile filaments.

Mitochondria and caveolae-like vesicles were commonly found in this cell (Figure 8D). Each cell was joined by hemidesmosome junctions.

Figure 8. Photomicrograph and transmission electron micrographs showing somatic sertoli cell (Sec) and perilobular myoid cell (Myc). (A) semithin section of somatic sertoli cell and perilobular myoid cell, (B-C) the characterization of somatic sertoli cell containing various types of residual bodies (Rb), laminar bodies (Lb), lipofuscin pigments (Lp), (D) the characterization of perilobular myoid cell containing caveolae-like vesicles (Clv), endoplasmic reticulum (ER), filaments (Fe), mitochondria (m).

DISCUSSION

Histology and histochemistry of testicular tissue. The testicular parenchyma consists of two parts, the germinal and interstitial compartments. The lobular testis in germinal compartment was the first reported in *R. brachysoma* in this study, quite similarly to Scombridae group such as *Thynnus thynnus* (Abascal *et al.*, 2004), *Scomber japonicus* (Nyuji *et al.*, 2012) and *Rachycentron canadum* (Brown-Peterson *et al.*, 2002). The testicular structure of this fish was also classified as an unrestricted spermatogonial type due to the presence of spermatogonia along the germinal compartment. It has been commonly observed in most teleost fish, except in the atherinomorphs

(Grier *et al.*, 1980; Grier, 1993; Parenti and Grier, 2004; Dietrich and Krieger, 2009). The detection of polysaccharides with PAS reaction in the interstitial compartment was presented in the *R. brachysoma*. It was suggested that they concerned the synthesis and secretion of sugars and proteins. Moreover, two main cell types were seen within the interstitial compartment, both perilobular myoid cell and steroidogenic Leydig cells. The perilobular myoid cells were also found similar to those of teleost fish (Billard, 1970; Grier *et al.*, 1989). Its functions concerned the contractile of the sperm in the environment during spawning within seminiferous lobules (Billard, 1970; Grier *et al.*, 1989). The steroidogenic Leydig cells were also mainly presented as cluster cells (about 4-5 cells) near blood vessels, as in other teleost fishes (Billard, 1983; Nagahama, 1983; U.S. EPA, 2006; Dietrich and Krieger, 2009). Its function has been reported in teleost fishes, including *Synbranchus senegalensis*, *Padogobius martensi* (Cinquetti and Dramis, 2003) and *S. marmotatus* (Lo Nostro *et al.*, 2004), which were investigated by histochemical analysis. All these studies were strongly positive to 3 β -hydroxysteroid dehydrogenase and were confirmed to produce the androgens inside the testis.

In the germinal compartments, this part was composed of various seminiferous lobules including somatic sertoli cells and spermatogenesis, in agreement with previous reports (Nagahama, 1983; Billard, 1992; Dietrich and Krieger, 2009). Additionally, seminiferous lobules of *R. brachysoma* also showed PAS positive, indicating that the secreting fluid may be composed of neutral glycoproteins, acid glycoconjugates, acid carboxylate and sialomucins (Santos *et al.*, 2010). The cytoplasmic processes of the somatic sertoli cells surrounded each spermatocyst as a cyst-like structure, containing many different stages of spermatogenesis (Nagahama, 1983; Billard, 1992; Dietrich and Krieger, 2009) as in *R. brachysoma*. As to the functions of somatic sertoli cells, they concerned with metabolites transport (Grier, 1975; Billard *et al.*, 1982) and phagocytosis of residual bodies and degenerating germ cells, including residual sperms (Grier, 1993; Grier and Taylor, 1998), although the characterizations of spermatogenesis like the spermatogenic stages (except for primary

spermatogonium and spermatozoa) were present within spermatocysts. The present result differed from García-López *et al.* (2005), who reported that only primary and secondary spermatogonia were present in the spermatocysts. At the final stage, spermatozoa were centrally located in the seminiferous lobules before they were released into environment, similar to that described in *Thunnus obesus* (Hashimoto *et al.*, 2003). This stage started the beginning of the fertilization.

Ultrastructure of spermatogenic stages.

Ultrastructural features of spermatogenic stages are interestingly seen in teleost fish (Fishelson *et al.*, 1990; Quagio-Grassiotto and Oliveira, 2008). Initially, the characterization of spermatogonium of this fish was large cell. Dense osmiophilic material or nuage was also normally occurred at this stage and scattered throughout the cytoplasm, as reported in different species of fishes such as *Poecilia reticulata* (Billard, 1984), *Oryzias latipes* (Hamaguchi, 1993) and *T. thynnus* (Abascal *et al.*, 2004). However, nuage structure in this species was the smallest structure and it could be the first found in primary spermatocyte, which was unclear throughout this research. It was possible that ultrasection (approximately 90 nm) in this study was still too thick, resulting in the loss of the details of spermatogonia stage. This particular feature involving origin and function are associated with the synthetic activity of spermatogenic step due to composing the ribonucleoproteins and mRNA (Hamaguchi, 1993; Lo Nostro *et al.*, 2003). After mitosis, the spermatogonia successively became primary spermatocytes. The characterization of the *R. brachysoma* primary spermatocytes was similar to other teleost fishes (Bhatti and Al-Daham, 1978; Brusle, 1981). The nucleus of primary spermatocyte contained high dense chromatin (compared to spermatogonia) and the synaptomemal complexes, particularly pachytene phase. This structure was basically presented in *Piaractus mesopotamicus* (Romagosa, 1991) and *T. thynnus* (Abascal *et al.*, 2004). On the other hand, secondary spermatocytes in *R. brachysoma* were rarely observed. This was similar to those found in several teleost fishes, probably due to their short-term appearance under rapidly dividing (Grier, 1976; Bhatti and Al-Daham, 1978; Brusle, 1981; Burke and Leatherland, 1984). Another

intercellular bridge, referred to as the cytoplasmic bridge was normally observed connecting during spermatogenic stage (Billard, 1984; Selman and Wallace, 1986). Their bridges were found through spermatocytes to spermatids in this fish.

During spermiogenesis, spermatids were differently seen under chromatin condensation and cytoplasmic organelles changes (Lahnsteiner and Patzner, 1990; Gwo *et al.*, 1993). The developmental pattern of spermatid in this fish could be prominently classified into three morphological stages; early, intermediate (referred to middle) and late spermatids, following the modified criterion from other family fish (Lahnsteiner and Patzner, 1990; Manni and Rasotto, 1997; Quagio-Grassiotto and Oliveira, 2008). At the beginning of spermiogenesis, the early spermatid of this fish was characterized to condense as granular-chromatin mass and the nucleus was completely condensed in the late spermatid. These features have been documented in teleost fish (Todd, 1976; Brusle, 1981; Sprando and Russell, 1988; Gwo *et al.*, 1993).

Finally, the pattern of spermatozoa morphology could be distinctly classified into two types based on the external and internal mode of the fertilization of the fishes (Jamieson and Grier, 1993; Jamieson, 2011). The spermatozoa ultrastructure of *R. brachysoma* had characteristic of that belonging to the primitive type, named aquasperm primitive type, which referred to as external fertilization, according to descriptions by Jamieson (2011), similar to *Makaira indica* (van der Straten *et al.*, 2006) and *T. thynnus* (Abascal *et al.*, 2002). It differed from internal fertilization type which had elongated head and complicated mid-piece structure (Lahnsteiner and Patzner, 1990). So, the type of spermiogenesis was established to type I and II in this present study, similar with the report by van der Straten *et al.* (2006). In *R. brachysoma*, the spermatozoon is considered as type II because of the shallowness of the nuclear fossa and nucleus outside the fossa. This type was evolutionarily more advanced than the type I sperm (Jamieson and Leung, 2011). A similar appearance had been described in scombrid group; Trichiuridae-*Trichiurus japonicus* (Hara and Okiyama, 1998), Scombridae-*S. australasicus* and *S. japonicus* (Hara and Okiyama, 1998), *S. japonicus* and *S. tritor* (Mattei, 1991) and *E. alletteratus* and *T. thynnus* (Abascal *et al.*, 2002). Other sperm

characterizations of *R. brachysoma* included the asymmetrical morphology of the head appeared as an ovoid nucleus with an acrosome-less in the anterior region, very similar to several scombrid species (Mattei, 1991; Hara and Okiyama, 1998) such as *T. thynnus* (Abascal *et al.*, 2002). It was well understood that its acrosome-less head was correlated with an egg micropyle (Romagosa *et al.*, 1999; Chung, 2008). Short mid-pieces were presented with several spherical mitochondria. This result agreed with the study in spermatozoon of *S. australasicus* (Hara and Okiyama, 1998), *Spiraea japonica* (Hara and Okiyama, 1998) and *T. thynnus* (Abascal *et al.*, 2002).

An asymmetric uniflagella was described here, similar to those described in most of the teleost species, *S. tritor* (Mattei, 1991), *E. alletteratus* (Abascal *et al.*, 2002), *S. australasicus* (Hara and Okiyama, 1998) and *S. japonicas* (Mattei, 1991; Hara and Okiyama, 1998). On the other hand, biflagella was only found in few teleosts, such as *Ictalurus punctatus* (Poirier and Nicholson, 1982), *Malapterurus* sp. (Mattei, 1988) and *Pimelodidae rhamdiasapo* (Jamieson, 2011). The flagellum of this species consisted of nine triplet's doubles of peripheral microtubules and the two central singlet microtubules. Thus, each axonemal pattern of the flagella in this fish was a typical 9+2 arrangement of microtubules as typically presented in primitive species (Romagosa, 1991).

Generally, the function of somatic sertoli cell concerned the nutrition, phagocytosis and steroidogenesis (Gresik *et al.*, 1973). The ultrastructure of sertoli cell was changed during the reproductive season, possibly that the materials within cytoplasm were correlated with reproductive function. A few glycogen particles were found, as in *Boleophthalmus pectinirostris*, in which these particles were concerned with spermatid nutrition during spermiogenesis (Chung, 2008). However, the study about nutrient during spermatids is unclear. Moreover, somatic sertoli cells in some fish displayed ultrastructural feature, where the change is related to gametogenic cycle (Cinquetti and Dramis, 2003). Testicular tissue was distinctly observed during regression as well as spermiation. Therefore, residual bodies normally observed, indicated the phagocytic function. This was also reported in several teleost (Grier *et al.*, 1980) as well as in mammals (Fawcett, 1975). Perilobular myoid

cells, which its function concerned the synthesis of the basement membrane (Skinner, 1991), did not degenerate during season. This is also found in many studies (Cinquetti, 1994; Cinquetti and Dramis, 2003). Moreover, contractile network could facilitate the ejection of sperm from the lobules during spawning (Grier *et al.*, 1980) because it exclusively contained the filamentous microfilament. This information was similar to our observations. Therefore, the basis of fine structure of perilobular myoid cell in this fish is directly correlated with its spermiation.

CONCLUSION

This study analyzed the histological and histochemical characteristics of the gonadal tissue in *R. brachysoma*, a commercially important marine fish in Thailand. The present result is considered the first report on the spermatogenic stage under light and electron microscopic levels from Thailand. It is also an important information for the phylogeny and systematics, which will be applied or used for the development of aquaculture technology.

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