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Ultrastructure of male germ cells in the testes of abalone, *Haliotis ovina* Gmelin

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Abstract

An ultrastructural study of male germ cells in the testes of *Haliotis ovina* revealed that spermatogenesis could be classified into 13 stages, based on the pattern of chromatin condensation and distribution of organelles, as follows: the spermatogonium; five stages of the primary spermatocyte; the secondary spermatocyte; five stages of the spermatid; and the spermatozoa. Each spermatogonium was round or oval, with a euchromatic nucleus and prominent nucleolus. The primary spermatocytes were divided into five stages: leptotene (LSc); zygotene (ZSc); pachytene (PSc); diplotene (DSc); and metaphase (MSc). The nucleus of the LSc contained scattered small heterochromatin blocks that were increasingly thickened in the ZSc. The PSc was characterised by a bouquet pattern of heterochromatin fibres. The DSc decreased in size, resulting in close clumping of chromatin blocks, whereas in the MSc, long and large blocks of chromosomes were formed and then moved to be aligned along the equatorial region. Secondary spermatocyte showed thick chromatin blocks that appeared reticulate. The spermatid could be divided into five stages ($St_{1–5}$). The St_1 was a large round cell and its nucleus contained homogeneous chromatin granules. In St_2, the nuclear chromatin started to condense into patches. The St_3 was smaller with a round nucleus containing dark blocks of heterochromatin. The St_4 became smaller still, with a round opaque nucleus. The St_5 was the smallest round cell, with almost completely condensed chromatin. The spermatozoon had a round to barrel-shaped head that contained completely condensed chromatin covered by a conical acrosome. The posterior border of the nucleus was flanked by five large spherical mitochondria and the tail consisted of axonemal microtubules surrounded by the plasma membrane.

Introduction

There are almost 100 species of abalone all belonging to the genus *Haliotis* (Fallu, 1991). These snails are widely distributed in tropical and temperate seas and inhabit submerged rock (Crofts 1929). Since ancient times, the abalone has been an economically important snail because of its decorative shell and food value. Abalone are commercially important molluscs in many countries, such as Japan, America, Mexico and Australia, where the culture of commercial abalone is well established. In Thailand, there are three species of abalone, namely *Haliotis ovina* Gmelin, 1791, *H. asinina* Linnaeus, 1758 and *H. varia* Linnaeus, 1929 (Nateewathana and Hylleberge 1986; Tookvinart *et al.* 1986; Bussarawit *et al.* 1990). Of these the three species, *H. ovina* is the one with economic potential (Nateewathana and Hylleberge 1986; Bussarawit *et al.* 1990).

Species of *Haliotis* are dioecious, with clear sexual dimorphism. The single testis envelops the digestive gland and, together, these structures form a large cone-shaped appendage called the conical organ, which wraps around the right and posterior margins of the shell muscle (Crofts 1929). The colour of the gonad indicates the sex of the abalone. The testis is cream to ivory, whereas the ovary is green in colour (Singhagraiwan 1989).

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There is no genital duct in *Haliotis* (Crofts 1929). The sperm from the testis are expelled into the cavity of the right renal organ, which is seen on the dorsal surface of the body and is overlapped by the digestive gland, and finally pass through shell perforations, which are located on the left side (Fallu 1991), into the water.

There have been extensive ultrastructural studies of male germ cells, especially the spermatozoa, in several haliotid species, such as *Haliotis rufescens* Swainson, 1822 (Lewis *et al.* 1980), *H. aquatilis* Reeve, 1846 (Shiroya and Sakai 1992), *H. diversicolor supertexta* Lischke, 1870 (Gwo *et al.* 1997), *H. discus* Reeve, 1846 (Sakai *et al.* 1982; Usui 1987), *H. midae* Linnaeus, 1758 (Hodgson and Foster 1992), *H. laevigata* Donovan, 1808 (Healy *et al.* 1998), and *H. asinina* (Apisawetakan *et al.* 2000; Sobhon *et al.* 2001). In general, spermatogenesis in these haliotid species can be classified into the following stages: spermatogonia; primary spermatocytes; secondary spermatocytes; spermatids; and spermatozoa. Sobhon *et al.* (2001) differentiated male germ cells in *H. asinina* into 14 stages based on the ultrastructure and pattern of chromatin condensation. These stages were the spermatogonium, six stages of the primary spermatocyte, the secondary spermatocyte, four stages of the spermatid and the spermatozoa (immature and mature). The general ultrastructure of haliotid spermatozoa is typical of the primitive type described for molluscs that reproduce by external fertilisation. The spermatozoa have a very simplified midpiece that is composed of a cluster of spherical mitochondria surrounding a pair of orthogonally arranged centrioles (Lewis *et al.* 1980; Sakai *et al.* 1982; Hodgson and Bernard 1986; Hodgson and Foster 1992; Shiroya and Sakai 1992; Gwo *et al.* 1997; Healy *et al.* 1998; Apisawetakan *et al.* 2000).

In the present study, we examined the ultrastructure of male germ cells in *H. ovina*, an abalone of potential economic importance, which is common along the coast of Thailand. The results are compared with those of *H. asinina* and other abalone species.

Materials and methods

Adult *H. ovina* (approximately 7 cm shell length, 170 g weight) were collected during June and July 1999 from Samed Island, Rayong Province, Thailand. A total of 20 male abalone was used in the present study. For the ultrastructural study, very small pieces of testes were fixed in 3% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.8) at 4°C overnight, washed in 0.1 M sodium cacodylate buffer and post-fixed in 1% osmium tetroxide in 0.1 M sodium cacodylate buffer for 1 h at 4°C. The pieces were then dehydrated in a graded series of ethanol, cleared using propylene oxide and infiltrated and embedded in Araldite 520 epoxy resin. Sections were cut with glass knives on a Sorvall MT-2 ultramicrotome. Semithin sections were stained with toluidine blue and observed with a light microscope. Ultrathin sections were stained with uranyl acetate and lead citrate and were viewed with a Hitachi H300 transmission electron microscope (TEM) operated at 75 kV.

Results

The male germ cells of *H. ovina* can be classified into 13 stages based on cell shape and size, nuclear shape and size, and pattern of chromatin condensation (Fig. 1). These stages comprise the spermatogonium, five stages of the spermatocyte, the secondary spermatocyte, five stages of the spermatid and the spermatozoa.

Spermatogonium

These cells lie on the outer edges of the lobe of the testis. The spermatogonium is spherical or oval, with a diameter of approximately 8–10 μm (Fig. 1*A*,*B*). The nucleus is round or slightly oval and contains mostly euchromatin, with only a thin rim of heterochromatin blocks attached to the inner surface of the nuclear envelope (Fig. 2*A*). The nucleolus is

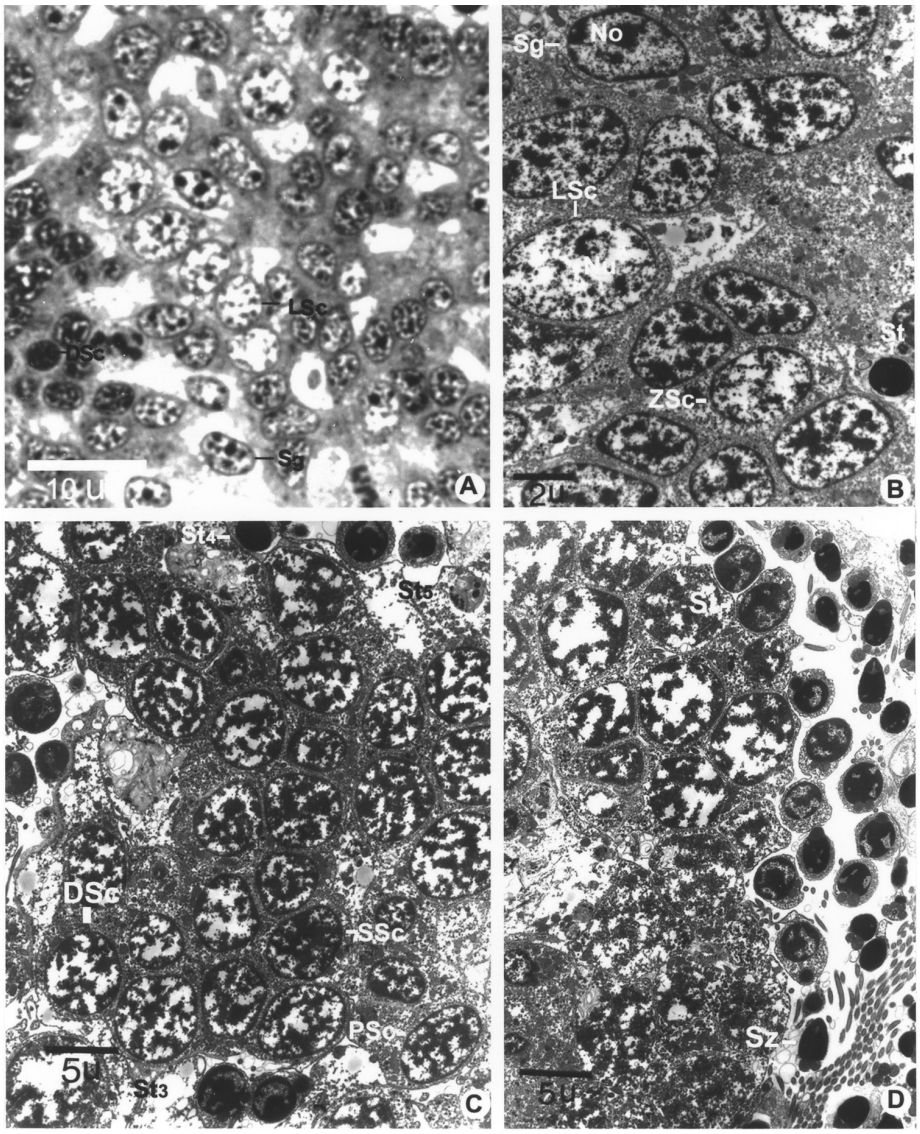

Fig. 1. Photomicrograph (*A*) and electron micrographs (*B–D*) showing various stages of the male germ cells of *Haliotis ovina*. Sg, Spermatogonia; LSc, leptotene primary spermatocyte; ZSc, zygotene primary spermatocyte; PSc, pachytene primary spermatocyte; DSc, diplotene primary spermatocyte; SSc, secondary spermatocyte; St, spermatid; Sz, spermatozoa.

prominent against the background of a rather transparent nucleoplasm. The cytoplasm contains free ribosomes and numerous mitochondria, which appear at the outer surface of the nuclear envelope (Fig. 2*A*).

Spermatocytes

The primary spermatocytes (PrSc) consist of five stages: leptotene (LSc), zygotene (ZSc), pachytene (PSc), diplotene (DSc) and metaphase (MSc) spermatocytes. The early cells

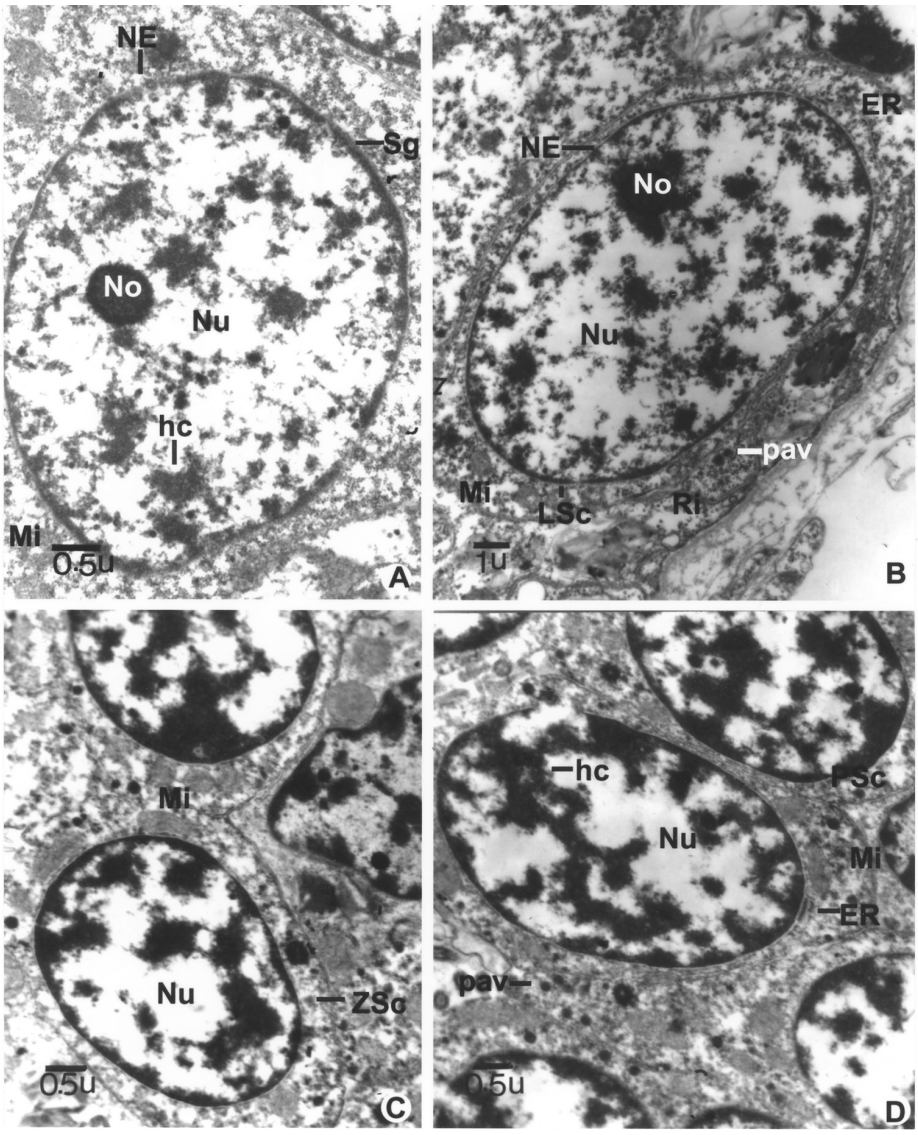

Fig. 2. *A*, High magnification of a spermatogonium (Sg) showing the round nucleus (Nu) with distinct nucleolus (No) and heterochromatin (hc) blocks. Mitochondria (Mi) are numerous on the outer surface of the nuclear envelope (NE). *B*, A leptotene primary spermatocyte (LSc) contains an oval nucleus with nucleolus. The cytoplasm contains mitochondria, ribosomes (Ri), endoplasmic reticulum (ER) and proacrosomal vesicles (pav). *C*, A zygotene primary spermatocyte contains an oval nucleus with dense heterochromatin and mitochondria in the cytoplasm. *D*, A pachytene primary spermatocyte (PSc) contains a nucleus with a bouquet pattern of heterochromatin. Note the presence of ER and mitochondria in the cytoplasm.

(LSc, ZSc, PSc) are round, approximately 12–15 μm in diameter, whereas the late-staged cells (DSc, MSc) are smaller in size (8–10 μm in diameter). Other distinctive differences among various stages of the PrSc are the pattern of chromatin condensation and the relative amount of euchromatin versus heterochromatin.

Leptotene spermatocyte

These cells are round, approximately 12–15 μm in diameter (Fig. 1*A*,*B*). The chromatin, which has started to condense into small blocks of heterochromatin, is scattered evenly throughout the nucleus. The nucleolus is still present, but not as prominent as that of the spermatogonium. The cytoplasm contains a few ribosomes, mitochondria and proacrosomal vesicles (Fig. 2*B*).

Zygotene spermatocyte

These cells are approximately the same size as the LSc (approximately 12 μm in diameter; Fig. 1*B*). Under TEM, the heterochromatin blocks are denser than those of the LSc and the nucleolus disappears (Fig. 2*C*). The cytoplasm contains proacrosomal vesicles and mitochondria, similar to those in the LSc.

Pachytene spermatocyte

These cells are round and their sizes are smaller than the LSc (approximately 10–12 μm in diameter; Fig. 1*C*). The heterochromatin appears as long threads and thick fibres that are entwined into a 'bouquet pattern', and are visible throughout the nucleus (Fig. 2*D*). The cytoplasm contains proacrosomal vesicles; the mitochondria and rough endoplasmic reticulum are greater in number than the LSc (Fig. 2*D*).

Diplotene spermatocyte

These cells are similar to the PSc, but are smaller (approximately 8–10 μm in diameter). The nucleus of the DSc is also smaller than that of the PSc. The chromatin strands, which are distributed among the denser nucleoplasm, become increasingly thicker than those in the earlier stage (Fig. 3*A*). The cytoplasm contains mitochondria and proacrosomal vesicles, similar to those in the PSc.

Metaphase spermatocyte

These cells (approximately 8 μm in diameter) exhibit thick chromosomes that are arranged close together along the equatorial region and the nuclear membrane completely disappears (Fig. 3*B*). The cytoplasm contains mitochondria, numerous ribosomes and proacrosomal vesicles (Fig. 3*B*).

Secondary spermatocyte

These cells are round and smaller than the MSc (approximately 6 μm in diameter; Fig. 1*C*). They are rarely observed and occur after meiosis I. The SSc shows thick chromatin blocks composed of criss-crossing fibres, which appear as reticulate chromatin (Fig. 3*C*). Fewer mitochondria and proacrosomal vesicles are visible in the cytoplasm (Fig. 3*C*).

Spermatids

There are five stages of spermatid ($St_{1–5}$), differing in size, nuclear shape and chromatin condensation pattern.

Spermatid I

The nuclei of St_1 are round (approximately 6 μm in diameter). The St_1 can be distinguished by their chromatin, which appears as homogeneous granules that are

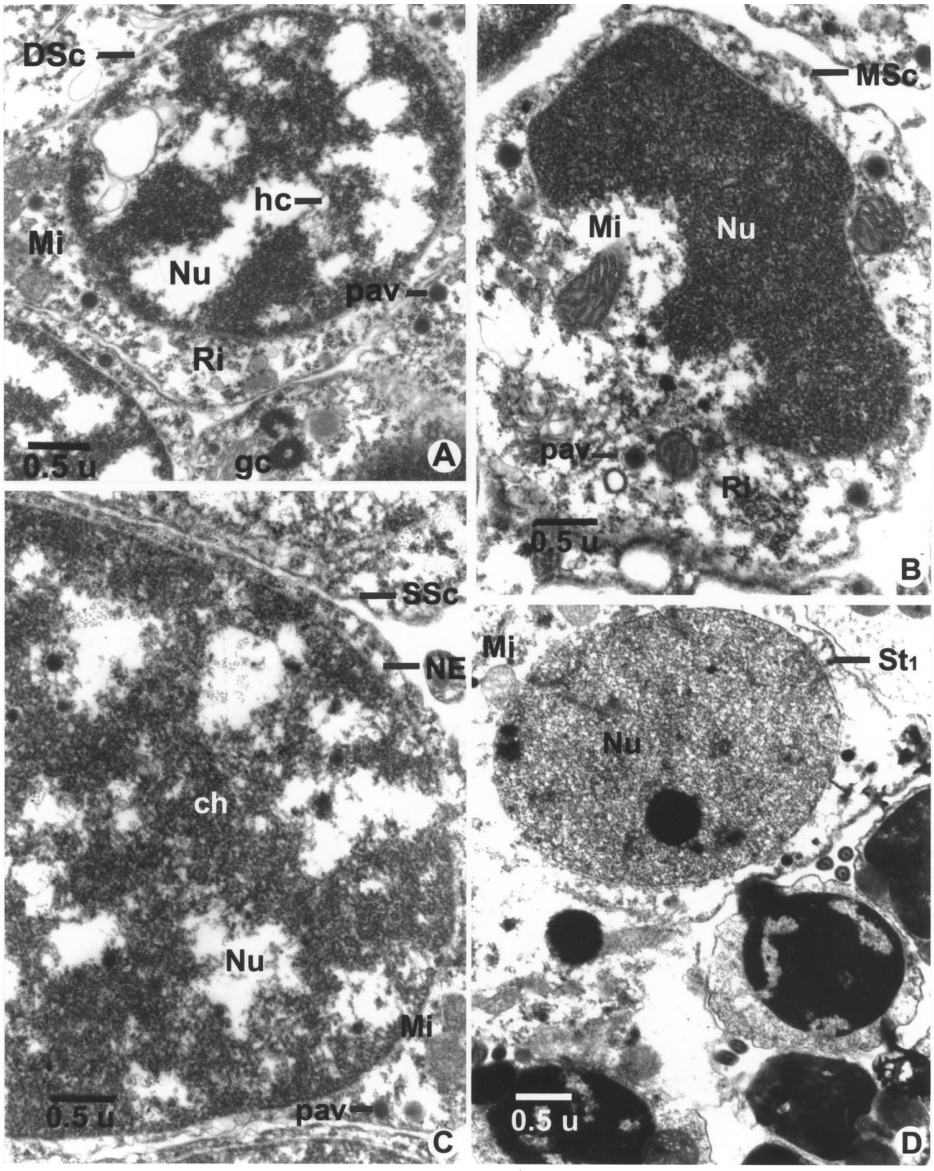

Fig. 3. *A*, A diplotene primary spermatocyte (DSc) contains a round nucleus (Nu) with very thick heterochromatin (hc). Note the presence of mitochondria (Mi), ribosomes (Ri), proacrosomal vesicles (pav) and Golgi body in the cytoplasm. *B*, A metaphase spermatocyte (MSc) exhibits thick chromosomes arranged along the equatorial region. Mitochondria, ribosomes and proacrosomal vesicles are still present. *C*, A secondary spermatocyte (SSc) with a round nucleus showing reticulate chromatin (ch). *D*, Spermatid I (St_1) showing a round nucleus with homogeneous granular chromatin. gc, Golgi complex.

uniformly spaced throughout the nucleus (Fig. 3*D*). As a result, the whole nucleus appears moderately dense without any intervening transparent area of nucleoplasm. The cytoplasm exhibits fewer organelles, such as mitochondria, which tend to be concentrated on one side of the nucleus (Fig. 3*D*).

Spermatid II

The general features of St_2 are similar to those of St_1, but the nucleus, which is still round, is smaller in size in St_2 (approximately 5 μm in diameter) and is located centrally within the cell (Fig. 4*A*). The chromatin appears as homogeneous granules that are uniformly spaced throughout the nucleus and condensed into patches. The cytoplasm contains a few mitochondria on one side and ribosomes (Fig. 4*A*).

Spermatid III

The St_3 is smaller than St_2 (approximately 4 μm in diameter) and the nucleus maintains a round shape. The chromatin begins to condense into dark blocks, with intervening light areas of nucleoplasm (Fig. 4*B*). The cytoplasm contains few ribosomes, mitochondria and proacrosomal vesicles (Fig. 4*B*).

Spermatid IV

The St_4 is smaller (approximately 3 μm in diameter), but still appears round in shape. The chromatin of St_4 is almost completely condensed, resulting in an opaque nucleus (Fig. 4*C*). At this stage, there is a continued loss of cytoplasm, a decrease in nuclear size and condensation of nuclear material. The cytoplasm contains numerous ribosomes, a few large mitochondria and centrioles (Fig. 4*C*).

Spermatid V

The St_5 is the smallest among ther spermiogenic cells (approximately 2 μm in diameter), but is still round in shape. The chromatin of St_5 is almost completely condensed (Fig. 4*D*). Fusion of proacrosomal vesicles into an acrosome that appears slightly indented at the anterior region of the nucleus can be seen (Fig. 4*D*). The cytoplasm contains numerous ribosomes and a few mitochondria (Fig. 4*D*). The mitochondria are fewer, but are larger in size, and occupy the posterior border of the nucleus (Fig. 4*D*).

Spermatozoa

Mature spermatozoa are detached from trabeculae and are arranged in rows further from the former stages, whereas their long tails are pointing outwards and are mingled with those of another spermatogenic unit (Fig. 1*D*). The nucleus (1 × 4 μm in size) is fully elongated and slightly tapered at the anterior end (Fig. 5*A*). The chromatin is completely condensed and the anterior portion of the head is covered by an acrosome that appears as an inverted cup, the concavity of which separates it from the anterior border of the indented nucleus (Fig. 5*A*). This subacrosomal space contains a crystalline acrosomal core embedded in more homogeneous material (Fig. 5*C*). The acrosomal matrix appears homogeneous, with varying degrees of electron opacity (Fig. 5*A*). The nuclear chromatin is completely condensed (Fig. 5*C*). The head of each spermatozoon comprises a barrel-shaped nucleus (Fig. 5*A*). At the posterior border of the nucleus, there are proximal and distal centrioles that are surrounded by a ring of five round mitochondria with cristae (Fig. 5*A*,*B*). The tail, or flagellum, consists of a 9 + 2 arrangement of microtubules and is surrounded by a plasma membrane (Fig. 5*D*).

Discussion

Most light microscopic studies on *Haliotis* have not categorised the various stages of spermatogenesis, apart from suggesting that there are four broad classes: spermatogonia,

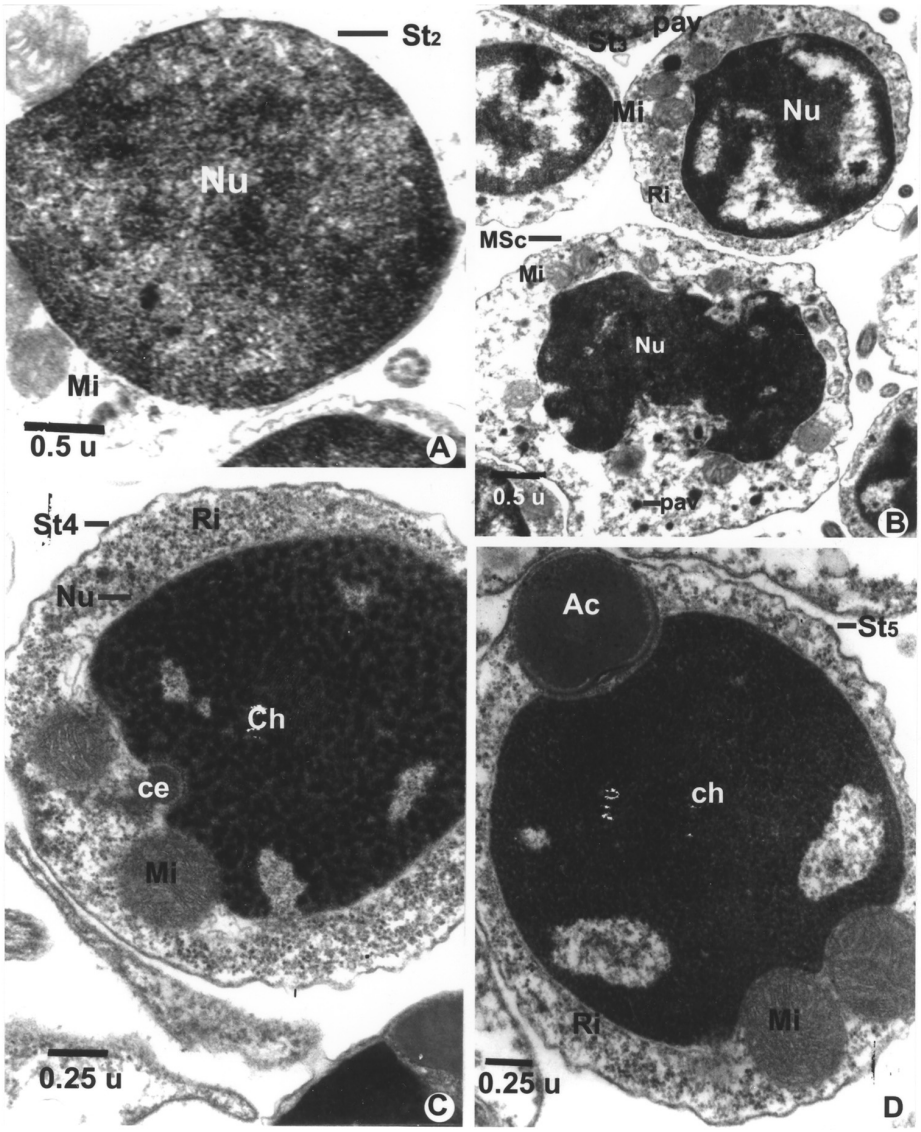

Fig. 4. *A*, Spermatid II (St_2) shows more condensed chromatin (ch) in a round nucleus (Nu). The cytoplasm exhibits fewer organelles. *B*, Spermatid III (St_3) contains a round nucleus. Chromatin is condensed into dark blocks. Mitochondria (Mi) begin to form a cluster. *C*, Spermatid IV (St_4) appears round in shape. The nucleus contains almost completely condensed chromatin. Mitochondria are located at the posterior border of the nucleus. Note the presence of centrioles (ce). *D*, Spermatid V (St_5) contains a nucleus with almost completely condensed chromatin. Note the fusion of proacrosomal vesicles (pav) into an acrosome (Ac). Mitochondria are larger and occupy the posterior border of the nucleus. Ri, Ribosome.

spermatocytes, spermatids and spermatozoa (Tomita 1967; Takashima *et al.* 1978). Apisawetakan *et al.* (1997) studied the gametogenic processes in *H. asinina* using the light microscope and classified spermatogenesis into 13 stages: spermatogonium, five stages of primary spermatocyte, secondary spermatocyte, four stages of spermatid and spermatozoa

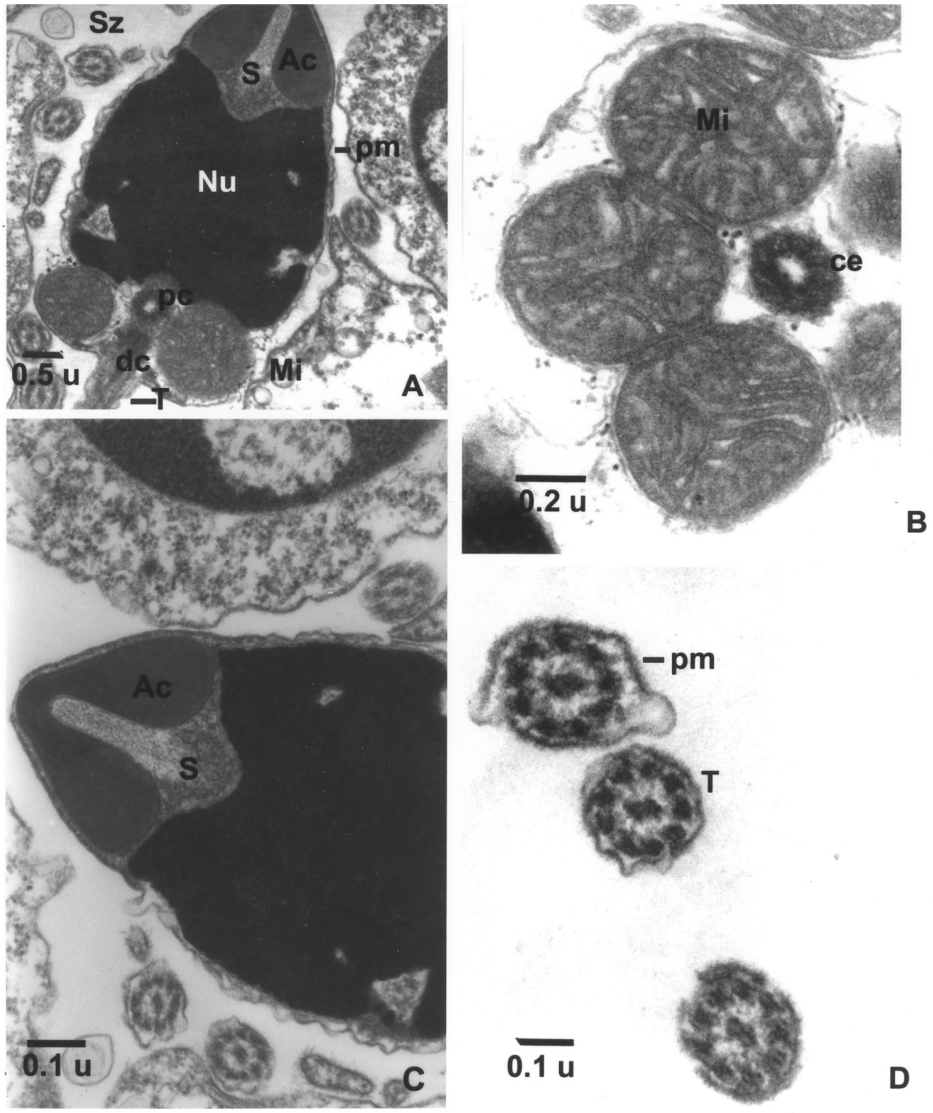

Fig. 5. Spermatozoa (Sz). *A*, Mature spermatozoa showing a fully elongated nucleus (Nu), acrosome (Ac) with subacrosomal space (S), proximal and distal centrioles (pc and dc, respectively) and a ring of mitochondria (Mi). *B*, High-power magnification of a ring of five mitochondria. *C*, High-power magnification of an acrosome. *D*, The tail (T) or flagellum consists of 9 + 2 arrangement of microtubules surrounded by a plasma membrane (pm). ce, Centriole.

(immature and mature). Sobhon *et al.* (2001) reported 14 stages of spermatogenesis based on their ultrastructural study: spermatogonium, six stages of primary spermatocyte, secondary spermatocyte, four stages of spermatid and spermatozoa (immature and mature). The present study determined 13 stages of spermatogenesis in *H. ovina*: the spermatogonium, five stages of the primary spermatocyte, the secondary spermatocyte, five stages of the spermatid and the spermatozoa. We could not observe the diakinetic stage of the primary spermatocyte, only the metaphase spermatocyte.

Like *H. asinina* (Sobhon *et al.* 1999, 2001), the spermatogonium of *H. ovina* is the earliest cell with a large nucleus containing a relatively large amount of euchromatin and a prominent nucleolus and little cytoplasm with free ribosomes and mitochondria. Spermatogonia divide mitotically to give rise to primary spermatocytes that pass through four stages of the first meiotic division. These prophase cells exhibit different forms of chromatin condensation. One remarkable characteristic of LSc, ZSc and PSc of both *H. ovina* and *H. asinina* is the presence of multiple proacrosomal vesicles in the cytoplasm. These vesicles begin to be synthesised in LSc and increase in PSc (Sobhon *et al.* 2001). In the MSc, the vesicles still appear quite numerous, whereas the chromatin becomes highly condensed into very large chromosomes. Thus, in this primitive gastropod, the proacrosomal vesicles are synthesised early in the LSc stage, even though they may be fused to form acrosomes much later in the late spermatid stages (Young and DeMartini 1970; Hodgson and Bernard 1986; Healy *et al.* 1998; Apisawetakan *et al.* 2000; Panasophonkul 2000; Sobhon *et al.* 2001). The fusion of multiple proacrosomal vesicles has been reported in several groups of vetigastropods, such as the trochids (Azevedo *et al.* 1985; Hodgson *et al.* 1990), bivalves (Hodgson and Bernard 1986) and scaphopods (Hou and Maxwell 1991). Although the very earliest stage of proacrosomal vesicle production was not observed in the present study, the ultimate source of these vesicles is undoubtedly the Golgi complex, as demonstrated previously for other vetigastropods (Azevedo *et al*. 1985; Healy and Harasewych 1992) and bivalves (Hodgson and Bernard 1986). Secondary spermatocytes, similar to those of *H. asinina* (Sobhon *et al.* 2001), have heterochromatin that exhibits a checkerboard pattern.

As mentioned earlier, there are five stages of spermatids in *H. ovina*, whereas Sobhon *et al.* (2001) described only four stages of spermatids in *H. asinina*. These classifications are based on size, chromatin granulation and condensation. Successive stages vary in size from 6 μm in St_1 to 2 μm in St_5. The major differences in the ultrastructure of developing spermatids of *H. ovina* and *H. asinina* are: (*1*) the formation of the acrosome begins in St_2 and is completed in St_3 for *H. asinina* (Sobhon *et al.* 2001), whereas this occurs at a later stage (St_5) in *H. ovina*; (*2*) all stages of spermatids in *H. ovina* retain a round shape, whereas they vary from round and oval ($St_{1–3}$) to ellipsoid (St_4) in *H. asinina* (Sobhon *et al.* 2001); (*3*) the acrosome of *H. ovina* appears slightly indented at the anterior region of the nucleus, whereas in *H. asinina* the acrosome only touches the nuclear envelope at the anterior end of the nucleus (Sobhon *et al.* 2001); and (*4*) the St_4 of *H. asinina* appears to be in a more advanced stage than that of *H. ovina* (i.e. there is a concentration of mitochondria in the posterior border of the nucleus and the centriole starts to form the axonemal complex of the tail (Sobhon *et al.* 2001); in *H. ovina*, only a concentration of mitochondria was found). There are two stages of spermatozoa ($Sz_{1–2}$) in *H. asinina* (Apisawetakan *et al.* 2000; Sobhon *et al.* 2001), whereas there is only one stage of spermatozoa in *H. ovina*. The Sz_1 is an immature spermatozoon with an acrosome and a short, developing tail, whereas Sz_2 is a mature spermatozoon.

The results of the present study show that the spermatozoa of *H. ovina* are very similar to those of *H. asinina*, except for the morphology of the sperm head. *Haliotis ovina* spermatozoa have a round to barrel-shaped head, whereas those of *H. asinina* are ellipsoid (Apisawetakan *et al.* 2000). In general, examination, to date, of spermatozoa in haliotid species shows that there are three types of sperm head: (*1*) short and globular or barrel-shaped (*H. ovina*; the present study); (*2*) ellipsoid (*H. laevigata* (Healy *et al.* 1998), *H. diversicolor supertexta* (Gwo *et al.* 1997) and *H. aquatilis* (Shiroya and Sakai 1992)); and (*3*) long and bullet-shaped (*H. discus* (Sakai *et al.* 1982; Usui, 1987), *H. rufescens*

(Lewis *et al.* 1980) and *H. midae* (Hodgson and Foster 1992)). Consequently, the sperm nuclei can also be classified into three types: round, ellipsoid and elongated. The chromatin of *H. ovina*, similar to that of *H. asinina* (Apisawetakan *et al.* 2000; Sobhon *et al.* 2001), appears to be of a granular type. This granular pattern of chromatin condensation can also be observed in other primitive gastropods, such as trochids (Hodgson *et al.* 1990; Healy 1996), scaphopods (Dufresne-Dube *et al.* 1993) and bivalves (Bozzo *et al.* 1993; Casas and Subirana 1994; Johnson *et al.* 1996). In *H. ovina* sperm, the acrosome is situated at the apex of the nucleus, as in *H. asinina* and also in many bivalves (Kubo 1977; Kubo and Ishikawa 1978; Apisawetakan *et al.* 2000; Sobhon *et al.* 2001).

The acrosome of *H. ovina* has an inverted cup shape, similar to those of *H. asinina*, *H. laevigata*, *H. diversicolor supertexta* and *H. aquatilis* (Gwo *et al.* 1997; Healy *et al.* 1998; Apisawetakan *et al.* 2000; Shiroya and Sakai 1992). In contrast, the acrosomes of *H. discus*, *H. rufescens* and *H. midae* are much longer and narrower (Lewis *et al.* 1980; Sakai *et al.* 1982; Hodgson and Foster 1992). The acrosomal core consists of a crystalline-like axis embedded within a moderately dense matrix that occupies the whole subacrosomal space. The core is much shorter. The crystalline material probably consists of actin filaments and associated proteins, as reported in other molluscs (Baccetti and Afzelius 1976; Shiroya *et al.* 1986; Tilney *et al.* 1987; Healy 1989). This acrosomal core may participate in the extension of the acrosomal process during the acrosomal reaction and fertilisation (Apisawetakan *et al.* 2000).

The tail of a *H. ovina* sperm consists of five globular mitochondria located at the posterior end of the nucleus surrounding a pair of centrioles. A long axoneme stretches backwards from the distal centriole, which is surrounded by mitochondria. The axoneme core consists of 9 + 2 doublets of microtubules surrounded by a plasma membrane. This type of tail and midpiece was also observed in *H. asinina* (Apisawetakan *et al.* 2000; Sobhon *et al.* 2001), *H. laevigata* (Healy *et al.* 1998), *H. aquatilis* (Shiroya and Sakai 1992), *H. diversicolor supertexta* (Gwo *et al.* 1997) and also in several other vetigastropods (Azevedo *et al.* 1985; Healy 1989; Hodgson *et al.* 1990; Healy and Harasewych 1992) and in many bivalves (Hodgson and Bernard 1990; Bozzo *et al.* 1993; Casas and Subirana 1994; Healy 1996; Johnson *et al.* 1996), all of which reproduce by external fertilisation. It is apparent that the sperm of those molluscs with external fertilisation do not require larger quantities of energy than those of molluscs with internal fertilisation. Such sperm usually have midpieces that contain large cylindrical or helical mitochondria (Jaramillo *et al.* 1986; Gallardo and Garrido 1989; Amor and Durfort 1990; Sretarugsa *et al.* 1991; Caceres *et al.* 1994).

Gwo *et al.* (1997) suggested that large species of Haliotidae usually possessed long sperm heads and small species contain short sperm heads. If this is the case, then *H. ovina* sperm should be classified into the latter group. Sperm of *H. ovina* bear certain similarities to those of *H. asinina*, except for the shape of the sperm head. Further studies need to be performed on the ultrastructure of haliotid sperm. We anticipate that continued comparative work in this field will help to shed further light not only on species relationships within the Haliotidae, but also on the validity of the many proposed subgenera.

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References

Amor, M. J., and Durfort, M. (1990). Changes in nuclear structure during eupyrene spermatogenesis in *Murex brandaris*. *Molecular Reproduction and Development* **25**, 348–356.

Apisawetakan, S., Thongkukiatkul, A., Wanichanon, C., Linthong, V., Kruatrachue, M., Upatham, E. S., Poomthong, T., and Sobhon, P. (1997). The gametogenic processes in a tropical abalone, *Haliotis asinina* Linnaeus. *Journal of the Science Society of Thailand* **23**, 225–240.

Apisawetakan, S., Sobhon, P., Wanichanon, C., Linthong, V., Kruatrachue, M., Upatham, E. S., Jarayabhand, P., Pumthong, T., and Nugranad, J. (2000). Ultrastructure of spermatozoa in the testis of *Haliotis asinina* Linnaeus. *Journal of Medical and Applied Malacology* **10**, 101–109.

Azevedo, C., Lobo-da-Cunha, A., and Oliveira, E. (1985). Ultrastructure of the spermatozoon in *Gibbula umbilicalis* (Gastropoda, Prosobranchia), with species reference to acrosomal formation. *Journal of Submicroscopic Cytology* **17**, 609–614.

Baccetti, B., and Afzelius, B. (1976). 'The Biology of the Sperm Cell'. Monograph of Developmental Biology, Vol. 10. (Karger: Basel.)

Bozzo, M. G., Ribes, E., Sagrista, E., Poquet, M., and Durfort, M. (1993). Fine structure of spermatozoa of *Crassostrea gigas* (Mollusca, Bivalvia). *Molecular Reproduction and Development* **34**, 206–211.

Bussarawit, S., Kawinlertwathana, P., and Nateewathana, A. (1990). Preliminary study on reproductive biology of the abalone. *Kasetsart Journal* (*Natural Science*) **24**, 529–539.

Caceres, C., Ribes, E., Muller, S., Cornudella, L., and Chiva, M. (1994). Characterization of chromatin-condensing protein during spermiogenesis in neogastropoda mollusc (*Murex brandaris*). *Molecular Reproduction and Development* **38**, 440–452.

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

Crofts, D. R. (1929). '*Haliotis*.' Liverpool Marine Biological Committee Memoir 29. (Liverpool University Press: Liverpool.)

Dufresne-Dube, J., Picheral, B., and Guerrier, P. (1993). An ultrastructure analysis of *Dentalium vulgare* (Mollusca, Scaphopoda) gametes with special reference to early events at fertilization. *Journal of Ultrastructural Research* **83**, 242–257.

Fallu, R. (1991). 'Abalone Farming.' (Blackwell Science: Oxford.)

Gallardo, C. S., and Garrido, O. A. (1989). Spermiogenesis and sperm morphology in the marine gastropod *Nucella crassilabrum* with an account of morphometric patterns of spermatozoa variation in the family Muricidae. *Invertebrate Reproduction and Development* **15**, 163–170.

Gwo, J.-C., Chang, H.-H., and Jong, K.-J. (1997). Ultrastructure of the spermatozoa of the small abalone, *Sulculus diversicolor supertexta* (Mollusca, Gastropoda, Haliotidae). *Journal of Submicroscopic Cytology and Pathology* **29**, 239–244.

Healy, J. M. (1989). Ultrastructure of spermiogenesis in the gastropod *Calliotropis glyptus* Watson (Prosobranchia: Trochidae) with special reference to the embedded acrosome. *Gamete Research* **24**, 9–19.

Healy, J. M. (1996). Molluscan sperm ultrastructure correlation with taxonomic units within the Gastropoda, Cephalopoda and Bivalvia. In 'Origin and Evolutionary Radiation of the Mollusca'. (Ed. J. Taylor.) pp. 99–113. (Oxford University Press: London.)

Healy, J. M., and Harasewych, M. G. (1992). Spermatogenesis in *Perotrochus quayanus* (Fischer and Bernardi) (Gastropoda: Pleurotomariidae). *The Nautilus* **106**, 1–14.

Healy, J. M., Beames, K. P., and Barclay, D. B. (1998). Spermatozoa of the Australian 'greenlip' abalone (*Haliotis laevigata* Donovan): ultrastructure and comparison with other gastropods, especially other Haliotidae (Vetigastropoda, Mollusca). *Invertebrate Reproduction and Development* **34**, 197–206.

Hodgson, A. N., and Bernard, R. T. F. (1986). Ultrastructure of the sperm and spermatogenesis of three species of Mytilidae (Mollusca, Bivalvia). *Gamete Research* **15**, 123–135.

Hodgson, A. N., and Foster, G. G. (1992). Structure of the sperm of some South African archaeogastropods (Mollusca) from the superfamilies Haliotidae, Fissurelloidea and Trochoidea. *Marine Biology* **113**: 89–97.

Hodgson, A. N., Heller, J., and Bernard, R. T. F. (1990). Ultrastructure of the sperm and spermatogenesis in five South African species of the trochid genus *Oxystele* (Mollusca, Prosobranchia). *Molecular Reproduction and Development* **25**, 263–271.

Hou, S. T., and Maxwell, W. L. (1991). Ultrastructural studies of spermatogenesis in *Antalis entalis* (Scaphopoda, Mollusca). *Philosophical Transactions of the Royal Society of London Series B* **333**, 101–110.

Jaramillo, R., Garrio, O., and Jorquera, B. (1986). Ultrastructural analysis of spermiogenesis and sperm morphology in *Cholrus giganteus* (Lesson, 1928) (Prosobranchia: Muricidae). *Veliger* **29**, 217–225.

Johnson, M. J., Casse, N., and Pennecc, M. (1996). Spermatogenesis in the endosymbiont-bearing bivalve *Loripes lucinalis* (Veneroida: Lucinidae). *Molecular Reproduction and Development* **45**, 476–484.

Kubo, M. (1977). The formation of a temporary-acrosome in the spermatozoon of *Laternula limicola* (Bivalvia, Mollusca). *Journal of Ultrastructural Research* **61**, 140–148.

Kubo, M., and Ishikawa, M. (1978). Organizing process of the temporary-acrosome in spermatogenesis of the bivalve *Lyonsia ventricosa*. *Journal of Submicroscopic Cytology* **10**, 411–421.

Lewis, C. A., Leighton, D. L., and Vacquire, V. D. (1980). Morphology of abalone spermatozoa before and after the acrosome reaction. *Journal of Ultrastructural Research* **72**, 39–46.

Nateewathana, A., and Hylleberge, J. (1986). A survey on Thai abalone around Phuket Island and feasibility study of abalone culture in Thailand. *Thai Fisheries Gazette* **39**, 177–190.

Panasophonkul, S. (2000). 'Spermatogenesis and chromatin condensation of male germ cells of a marine oyster, *Saccostrea forskali* Gmelin.' MSc Thesis. (Mahidol University: Bangkok, Thailand.)

Sakai, Y. T., Shiroya, Y., and Haino-Fukushima, K. (1982). Fine structural changes in the acrosome reaction of the Japanese abalone, *Haliotis discus*. *Development*, *Growth and Differentiation* **24**, 531–542.

Shiroya, Y., and Sakai, Y. T. (1992). Coiled filamentous structure in the sperm acrosome of Gastropoda, *Sulculus aquatilis* and *Turbo cornutus*. *Development*, *Growth and Differentiation* **34**, 509–516.

Shiroya, Y., Hosoya, H., Mabuchi, I., and Sakai, Y. T. (1986). Actin filament bundle in the acrosome of abalone spermatozoa. *Journal of Experimental Zoology* **239**, 105–115.

Singhagraiwan, T. (1989). 'The experiment on breeding and nursing of donkey's ear abalone (*Haliotis asinina* Linne).' (The Eastern Marine Fisheries Development Center, Marine Fisheries Division, Department of Fisheries, Ministry of Agriculture and Cooperatives: Thailand.)

Sobhon, P., Apisawetakan, S., Chanpoo, M., Wanichanon, C., Linthong, V., Thongkukiatkul, A., Jarayabhand, P., Kruatrachue, M., Upatham, E. S., and Pumthong, T. (1999). Classification of germ cells, reproductive cycle and maturation of gonad in *Haliotis asinina* Linnaeus. *Science Asia* **25**, 3–21.

Sobhon, P., Apisawetakan, S., Linthong, V., Pankao, V., Wanichanon, C., Meepool A., Kruatrachue, M., Upatham, E. S., and Poomthong, T. (2001). Ultrastructure of the differentiating male germ cells in *Haliotis asinina* Linnaeus. *Invertebrate Reproduction and Development* **39**, 55–66.

Sretarugsa, P., Ngowsiri, U., Kruatrachue, M., Sophon, P., Chavadej, J., and Upatham, E. S. (1991). Spermiogenesis in *Achatina fulica* as revealed by electron microscopy. *Journal of Medical and Applied Malacology* **3**, 7–18.

Takashima, F., Okuno, M., Nichimura, K., and Nomura, M. (1978). Gametogenesis and reproductive cycle in *Haliotis diversicolor diversicolor* Reeve. *Journal of the Tokyo University of Fisheries* **1**, 1–8.

Tilney, L. G., Fukui, Y., and Derosier, D. J. (1987). Movement of the actin filament bundle in *Mytilus* sperm: a new mechanism is proposed. *Journal of Cell Biology* **104**, 981–993.

Tomita, K. (1967). The maturation of the testis of the abalone, *Haliotis discus hannai* Ino, in Rebun Island, Hokkaido, Japan. *Scientific Report of Hokkaido Fisheries Experimental Station* **7**, 1–7.

Tookvinart, S., Leknim, W., Donyadol, Y., Preeda-Lampabutra, Y., and Perngmak, P. (1986). 'Survey on species and distribution of abalone (*Haliotis* spp.) in Surajthani, Nakornsrithammaraj and Songkhla province.' (National Institute of Coastal Aquaculture, Department of Fisheries, Ministry of Agriculture and Cooperatives: Thailand.)

Usui, N. (1987). Formation of the cylindrical structure during the acrosome reaction of abalone spermatozoa. *Gamete Research* **16**, 37–45.

Young, J. S., and DeMartini, J. D. (1970). The reproductive cycle, gonadal histology and gametogenesis of the red abalone, *Haliotis rufescens* (Swainson). *California Fish and Game* **56**, 298–309.