THE GAMETOGENIC PROCESSES IN A TROPICAL ABALONE, *HALIOTIS ASININA* LINNAEUS

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ABSTRACT

The gonad histology and gametogenic processes of a species of abalones found along the coast of Thailand, Haliotis asinina, were studied by light microscopy using special stainings. The outer gonadal wall is similar in both sexes, and consists of fibro-muscular tissue forming a capsule-like structure. This capsule forms connective tissue trabeculae that partition the gonad into compartments, where gonial and early germ cells are attached to each trabecula to form oogenetic or spermatogenic unit. Within the connectives of trabeculae are vessels that contain haemolymph. Cells in oogenetic process could be classified into six stages according to their histological characteristics: oogonium, and five stages of oocytes, ie., with light basophilia (I), with intense basophilia and oil droplets (II), with primary yolk granules (III), with secondary yolk granules and thin jelly coat (IV), and mature ovum with 2 types of yolk granules and fully formed jelly coat (V). The cells in spermatogenetic process could be classified into thirteen stages: spermatogonium, five stages of primary spermatocytes, secondary spermatocyte, four stages of spermatids and two stages of spermatozoa.

INTRODUCTION

Abalones are important economic animals in many countries, such as Japan, America, Mexico and Australia where commercial abalone farms are well established. In Thailand, there are three species of abalones along the coast, which are *Haliotis asinina, Haliotis ovina* and *Haliotis varia*[1,2]. Among the three species, *Haliotis asinina* has the largest size and the most economic potential because of their maximum proportion of flesh. In 1991, the Coastal Development Centre in Rayong Province had been successful in increasing the fecundity of this species and the production of larvae by artificial fertilization, however the induction of artificial spawning had not been successful[3]. The histology of the genital organ and gametogenic processes have been studied in other temperate species, such as *Haliotis discus hannai* Ino, in Rebun Island, Hokkaido, Japan[4,5], *Haliotis diversicolor diversicolor* Reeve[6]. By contrast, similar information on *Haliotis asinina,* which is a tropical abalone, is still lacking. The basic knowledge concerning the cellular details in the gonads, particularly with regard to the optimal time that the gonads exhibit full maturation and readiness to be artificially spawned, will contribute to the improvement of the spawning efficiency that could be applied in the aquaculture system of this species in Thailand. The present study is thus focused on the histology of the gonads

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and the classification of cells in gametogenic processes of adult *Haliotis asinina* in both sexes by light microscopy using paraffin and semithin methods.

MATERIALS AND METHODS

Mature abalones, aged at least 36 months, were collected monthly from the land-based culture system at the Coastal Aquaculture Development Centre, Prachuapkhirikhun Province. The fixed gonads were prepared for light microscopic observations by the paraffin and semithin methods, and observed under an Olympus Vanox microscope.

Paraffin method

The abalones were anesthetised in 5% magnesium chloride for one hour. The gonads were dissected out and fixed in Bouin's fixative for overnight, dehydrated in graded series of ethanol, cleared with dioxane, infiltrated and embedded in paraffin, sectioned at 5-micron thick, and finally stained with Hematoxylin-Eosin (H&E), and PAS.

Semithin method

The gonads were dissected out and sliced into very small pieces, while simultaneously fixed in a solution of 3% glutaraldehyde in 0.1M sodium cacodylate buffer, pH 7.4, at 4°C. The fixation was continued for overnight, followed by washings in 0.1M sodium cacodylate buffer for removal of the fixative. The specimens were then post-fixed in 1% osmium tetroxide in 0.1M sodium cacodylate buffer for one hour at 4°C, then dehydrated in graded series of ethanol, cleared in propylene oxide, infiltrated and embedded in Araldite 502 resin, and finally polymerized at 45°C and 60°C for 48 hours each. Blocks of specimens were sectioned at 1-micron thickness in Porter Blum MT-2 ultramicrotome, and stained with Methylene Blue.

RESULTS

Gross Anatomy (Fig.1A)

A fully grown *H. asinina* has an oval shape, whose shell is brownish-green in color. The average shell length, *ie.*, the anterior-posterior axis, is about 8-10 cm, and the width is about 4 cm, with an average maximum weight about 170 gm. Fecundity is observed in females with shell length of at least 4.4 cm, while sexually matured males are at a smaller size than females, with shell length about 3.1 cm. In each animal, a single gonad envelopes the hepatopancreas, and together they form a large cone-shaped appendage called conical organ, wrapping around the posterior margin of the shell muscle. The color of gonad indicates the sex of an abalone: it is creamy to yollow in color in male, grey-green to green in female. The gonads of both sexes are opened through a simple longitudinal slit in the roof of the central part of the right renal organ. Eggs and sperm are expelled into the cavity of the right renal organ, which is overlapped dorsally by the hepatopancreas, and they finally pass through the shell perforations.

Gonadal Histology

Cross sections of conical organ exhibit hepatopancreas surrounded by the testis or ovary (Fig.1B,C). At the base of the conical organ hepatopancreas appears large and occupies most of the cross sectional profile (Fig.1C), while it becomes smaller towards the tapered end of the organ (Fig.1B).

Both testis and ovary are surrounded by thin capsules made of dense collagenous fibers mixed with muscle cells. The connective tissue from capsule extends into the interior of the gonads to form flat sheets of trabeculae that are connected at their innermost ends to the thin loose capsule of hepatopancreas (Fig.1B,C). As a result the gonads are divided into small compartments. Within the connectives of trabeculae, there are small vessels running through their whole course (Fig.2A). These may be capillaries that are branching out from the larger subcapsular vessles. Around the capillaries, there are loosely packed collagen fibers mixed with numerous small cells exhibiting dense ellipsoid nuclei. Some of them may be fibroblasts while others may be follicular or supporting cells that surround the early stage germ cells (Fig.4A,B,D).

Each trabecula forms the axis on which germ cells are attached. Early stage cells, such as spermatogonia and oogonia, are seen closely bound to the connectives of trabeculae. Middle stage germ cells, such as spermatocytes and oocytes, are more detached and appear further from trabeculae; while mature cells, such as spermatozoa and stage V oocytes, are completely detached and appear in the lumen of the compartments. Such appearance give rises to a discrete gametogenic unit, representing perhaps a single clone of germ cells that may arise from a single group of gonial cells. These clones are termed spermatogenic or oogenetic unit (Fig.2B,C).

Classification of germ cells

Spermatogenic cells: Male germ cells, as observed by light microscopy, in both paraffin and plastic-embedded semithin sections could be classified into 13 stages (Fig.3A-D; TextFig.1).

Spermatogonium (Sg): Sg is a spherical or oval-shaped cell with diameter around 10-12 mm. Its nucleus is round or slightly indented with diameter about 6-7 μm. The nucleus contains mostly euchromatin with only a thin rim of heterochromatin attached to the inner surface of nuclear envelope. The nucleolus is prominent and stands out from the rather transparent nucleoplasm.

Primary spermatocytes (PrSc): PrSc consists of 5 stages, *ie.*, leptotene (LSc), zygotene (ZSc), pachytene (PSc), diplotene (DSc) and metaphase (MSc) stages. The early cells are round and become increasingly larger ranging from 12-15 μm. Then they are gradually decreased in size from DSc to MSc, which are about 7-10 μm, with nuclear size about 4-6 μm. Another distinctive difference among various stages of PrSc is the pattern of chromatin condensation and the relative amount of euchromatin versus heterochromatin. In LSc heterochromatin appears as small blocks that are evenly scattered throughout the nucleus. The nucleolus is still present but not as prominent as in Sg. In ZSc blocks of heterochromatin increase in size and density, and the nucleolus disappears. In PSc heterochromatin appears as long threads that are entwined into "bouquet pattern". In DSc these strands become increasingly thicker and the nucleoplasm appears denser than in earlier stages. In MSc pairs of chromatids become aligned along the equatorial region, and the nuclear membrane completely disappears.

Secondary spermatocyte (SSc): SSc is a small round cell about 6 μm in diameter, with a nucleus about 4 μm. However, they are rarely observed , and when present they exhibit thick cords of heterochromatin that are crossing one another, appearing as X and Y figures.

Spermatids (St): There are 4 stages of spermatids, depending on the size, chromatin granulation and condensation. All stages are round and ranging in size from 6 μm in St_1 to 3 μm in St_4. St_1 is larger than St_2, however their chromatin appear similar in density and consist of fine granules that are uniformly distributed throughout the nucleus. As a result the whole nuclei appear moderately dense without any intervening transparent area of nucleoplasm. In St_3 the cell becomes smaller and the chromatin is condensed into dark blocks with intervening

light areas of nucleoplasm. In St_4 the cell becomes smallest but still appears round. Its chromatin becomes completely dense, thus the nucleus appears opaque.

Spermatozoa (Sz): There are 2 stages of Sz: Sz_1 is an immature spermatozoon that begins to show elongated nucleus whose chromatin is completely dense like in St_4. There is a clear cap-like structure apposing on one side of the nucleus, which could be maturing acrosome. The tail is short and still forming. In mature spermatozoa (Sz_2) the nucleus is fully elongated, with the size about 1.5 x 3 μm, and the chromatin is completely dense. The heads of Sz_2 are embedded in the cytoplasm of supporting cells while their long tails are pointing outwards and mingled with those from another spermatogenic unit.

Oogenetic cells: There are 6 stages of female germ cells, including one stage of oogonium and 5 stages of oocytes (Fig.4, 5; TextFig 1).

Oogonium (Og): Og is a round or oval-shaped cell (Fig.4A,B,C), whose size is about 10-12 μm. Its nucleus is round and about 7 μm in diameter. It contains small blocks of heterochromatin attached to the inner surface of nuclear envelope, with the remaining majority appearing as euchromatin. The nucleolus is present but may not be as prominent as in Sg. The cytoplasm is stained light blue by H&E and methylene blue, which implies its basophilic property. Og are attached to the capsular side of trabeculae, and usually are concentrated in groups (Fig.4A,B,C). Each Og is surrounded by flat, squamous-shaped follicular cells.

Stage 1 oocyte (Oc_1): Oc_1 is a round or scallop-shaped cell that is closely adhered to the trabecula. It is about 24 μm in size, with a round nucleus about 12 μm in diameter. The nucleolus is present but tends to be obscured by the rather dense chromatin and nucleoplasm. Similarly, the boundary of the nuclear envelope is not clearly discernible. The cytoplasm is stained deep blue with H&E in paraffin and methylene blue in semithin sections, which indicates its intense basophilic property (Fig.4A,B,D; 5A,B,C; 6B). Each Oc_1 is surrounded by few follicular cells.

Stage 2 oocyte (Oc_2): Oc_2 becomes larger and transforms into columnar or flask-shape, with the cell size around 30 x 55 μm, and nuclear size about 22 μm. It is still attached to the connective of trabecula by the narrow part, and each Oc_2 is surrounded by several follicular cells. The nucleolus and nuclear membrane boundary are clearly distinct due to the more transparent nucleoplasm and the presence of mostly euchromatin. In some cells the chromatin appear as long intertwined and zig-zag strands (Fig.4C,D). The cytoplasm is stained light blue similar to Og, and contains clusters of clear lipid droplets (Fig.4B,D).

Stage 3 oocyte (Oc_3): This cell becomes increasingly larger and assumes a pear shape, with the narrow side or base still attached to the connective of trabecula. The cell size is about 35-70 μm, with the nuclear size about 20 μm. The nucleus contains mostly euchromatin, and the nucleoplasm is quite transparent. The nucleolus is distinct and becomes enlarged due to the uncoiling of nucleolar chromatin. In addition to clear lipid droplets, the cytoplasm also contains reddish yolk platelets (in H&E stained sections). Fine blue granules are evenly distributed between lipid droplets and yolk platelets (Fig.5A; 6A). Follicular cells surround both the cell body and its base near trabecula.

Stage 4 oocyte (Oc_4): This cell is large and assumes a polygonal shape. Some are still attached to trabeculae by slender cytoplasmic processes. The cell size is about 60-80 μm, with nuclear size about 35 μm. The nucleus contains mostly euchromatin and transparent nucleoplasm. Hence the nucleolus is clearly visible, and it also becomes enlarged and more transparent in comparison to earlier stages. The cytoplasm is filled with reddish yolk platelets and a few lipid droplets. Fine blue-stained granules are decreased in central area of the cytoplasm,

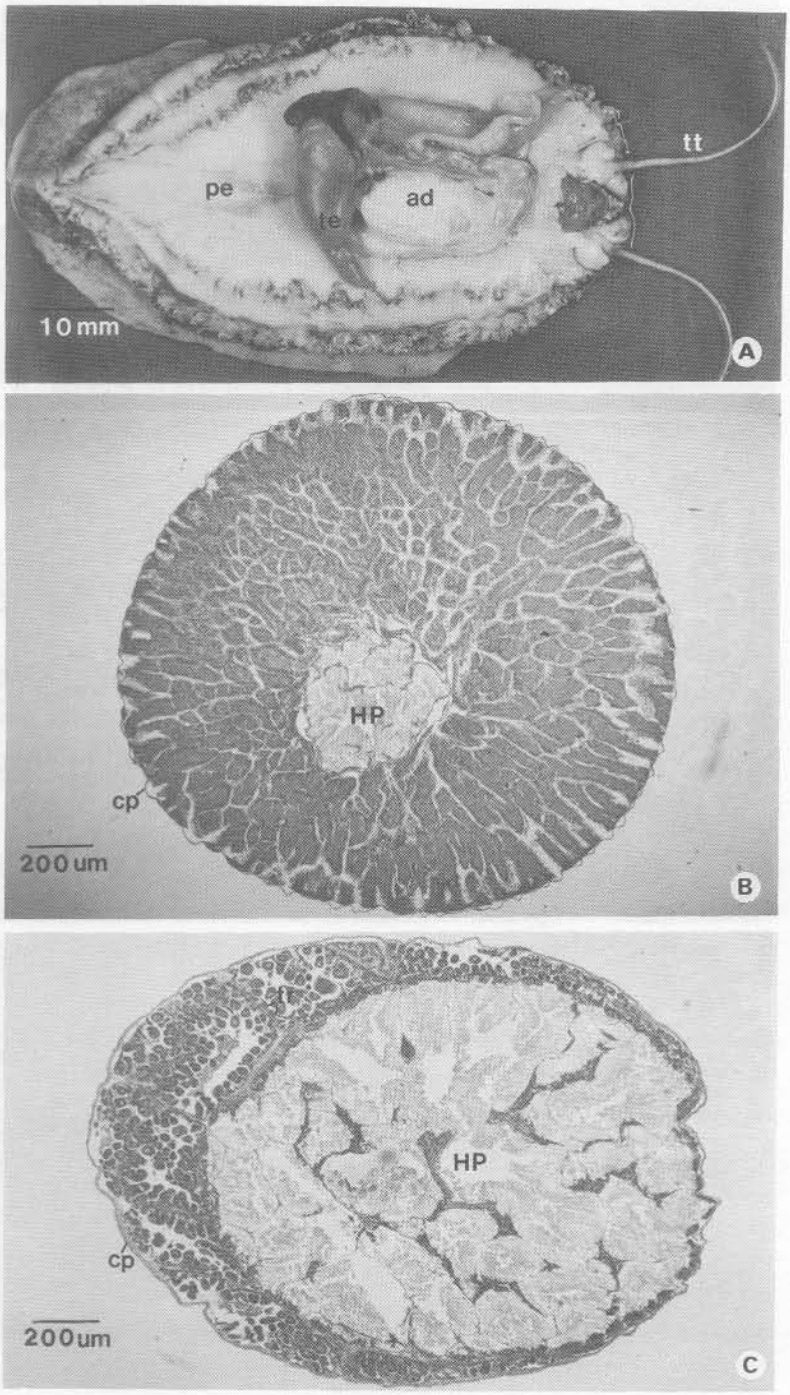


Fig.1 A) The dorsal view of a shell-freed male abalone, showing testis (te), adductor muscle (ad), pedal muscle (pe), and tentacle (tt).

B) Cross section of the testis, showing hepatopancreas (HP) surrounded by testicular tissue which is, in turn, surrounded by thin connective tissue capsule (cp).

C) Cross section of the ovary, showing hepatopancreas (HP) surrounded by ovarian tissue and fibrous capsule (cp).

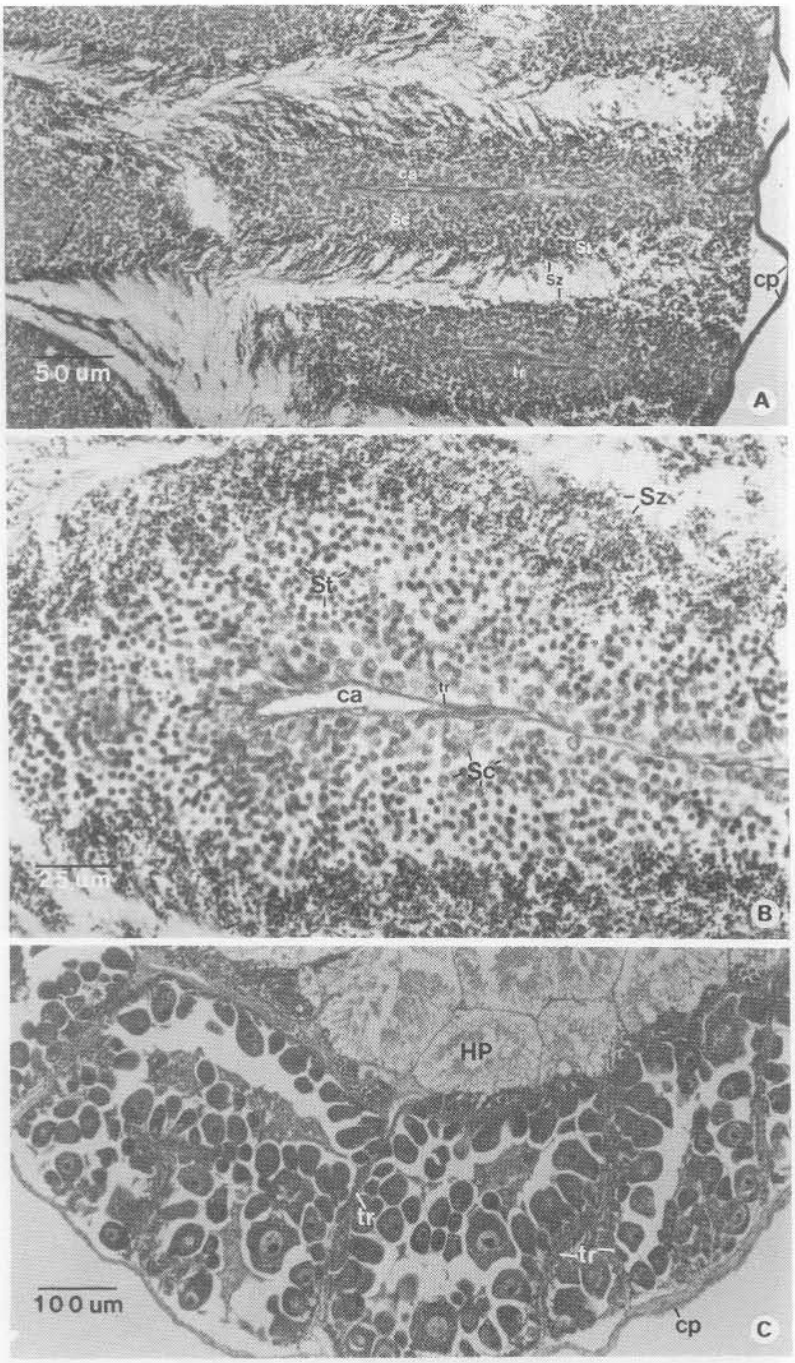


Fig.2 A,B) A spermatogenic unit consists of a central trabecula (tr) arising from capsule (cp-in A), surrounded by various stages of germ cells. A capillary (ca) is present inside each trabecula, and successive maturing stages of germ cells lie at different distance from the connective of trabecula (Sc-spermatocyte, St-spermatid, Sz-spermatozoa).

C) An oogenetic unit also consists of an axis of trabecula (tr) surrounded by early stages of oocytes (I-III). Late stages oocytes (IV, V) are released into the central area of the compartment partitioned off by adjacent trabeculae.

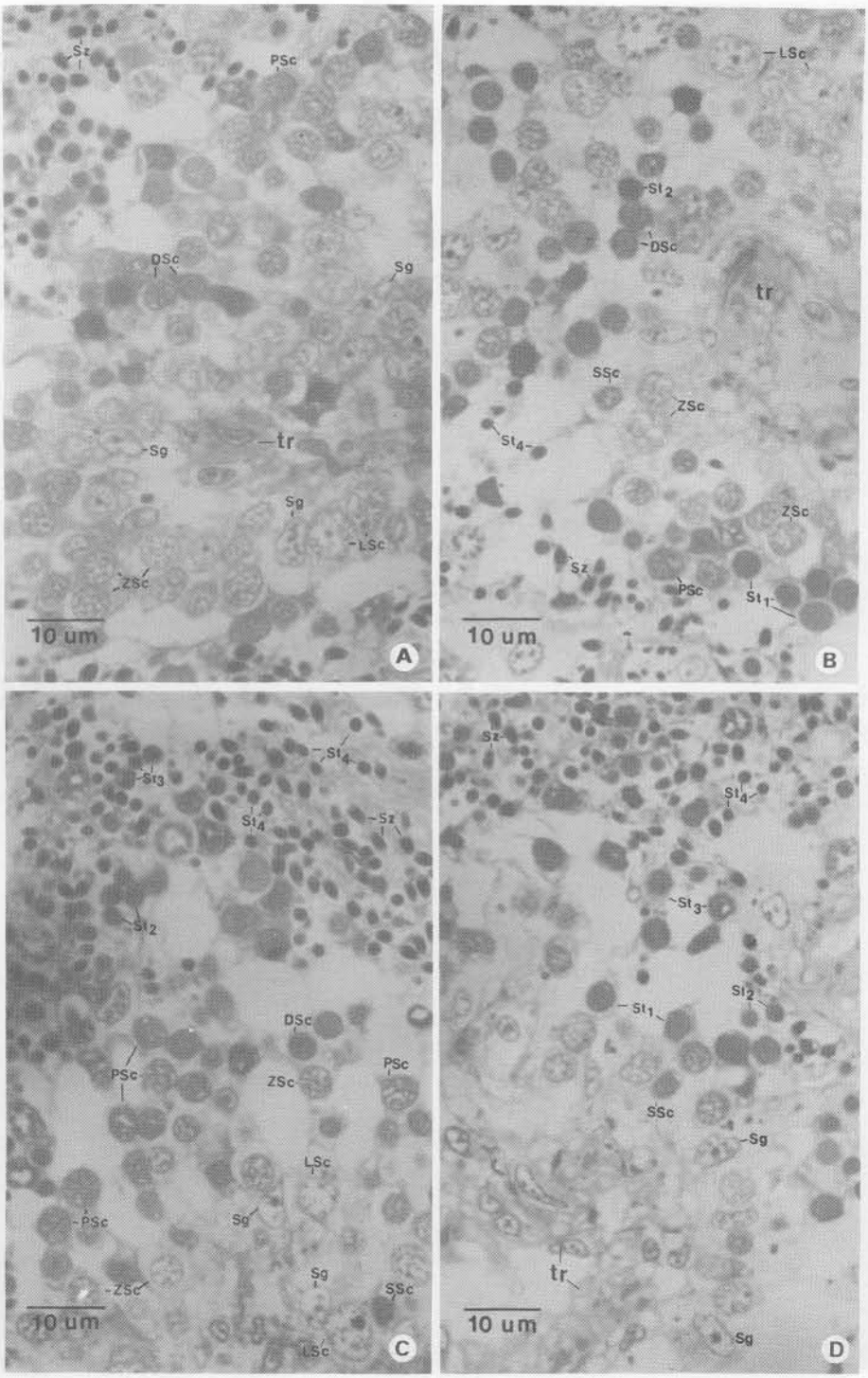


Fig.3 A-D) Semithin sections demonstrating various stages of male germ cells. Lying closest to each trabecula (tr) are spermatogonia (Sg), different stages of primary spermatocytes (LSc-leptotene; ZSc-zygotene; PSc-pachytene; DSc-diplotene; MSc-metaphase), and further away are secondary spermatocytes (SSc), various stages of spermatids (St_{1-4}), and on the outermost periphery of a spermatogenic unit are spermatozoa (Sz_{1-2}).

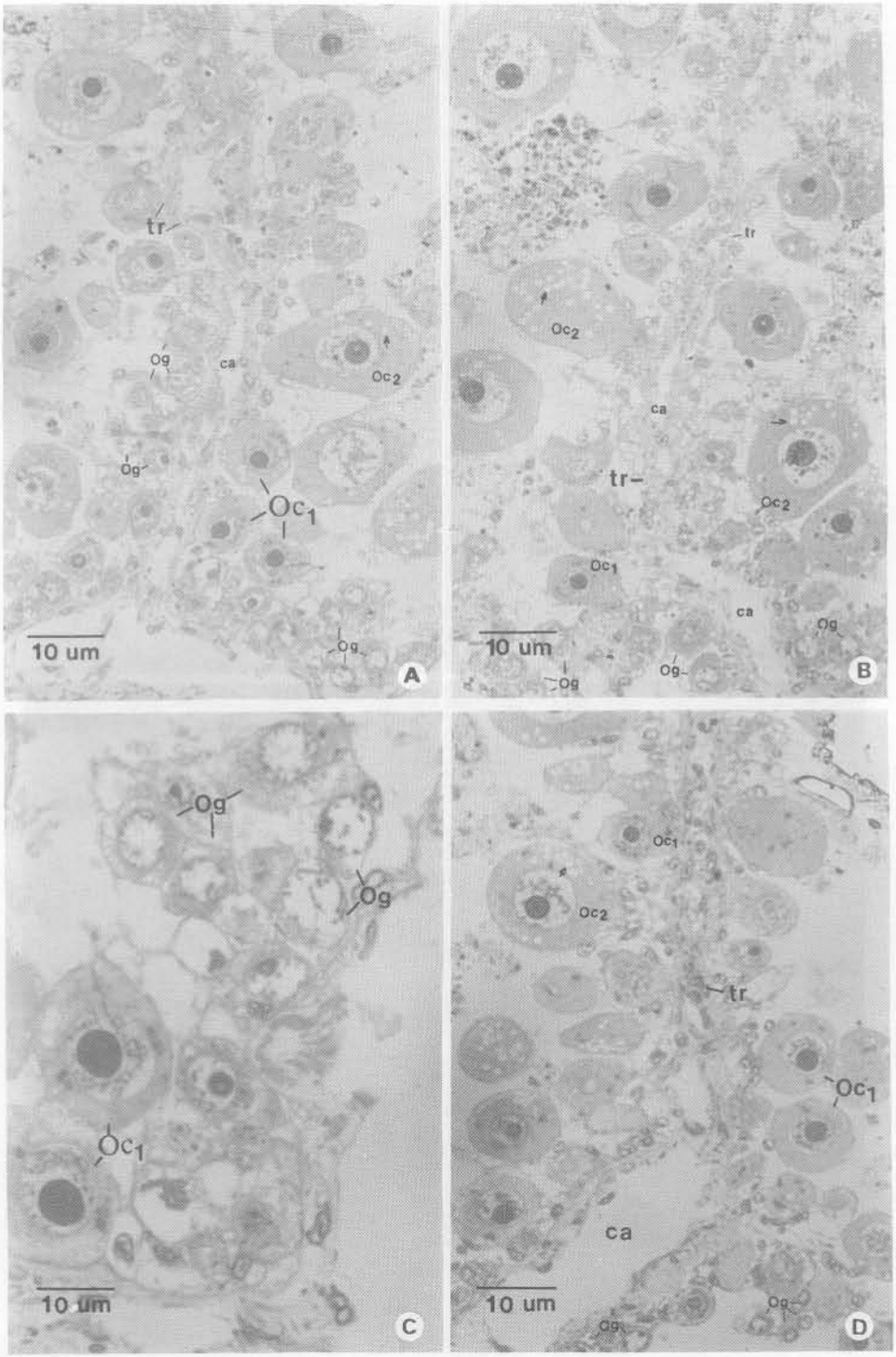


Fig.4 A-D) Semithin sections demonstrating various stages of oocytes surrounding each trabecula (tr), which contains a capillary (ca) in the center. Closely attached to the connective of trabecula are oogonia (Og), stage I-III oocytes (Oc_1-Oc_3). In A and D there are clusters of oogonia lying close in the areas where trabeculae arise from the capsule.

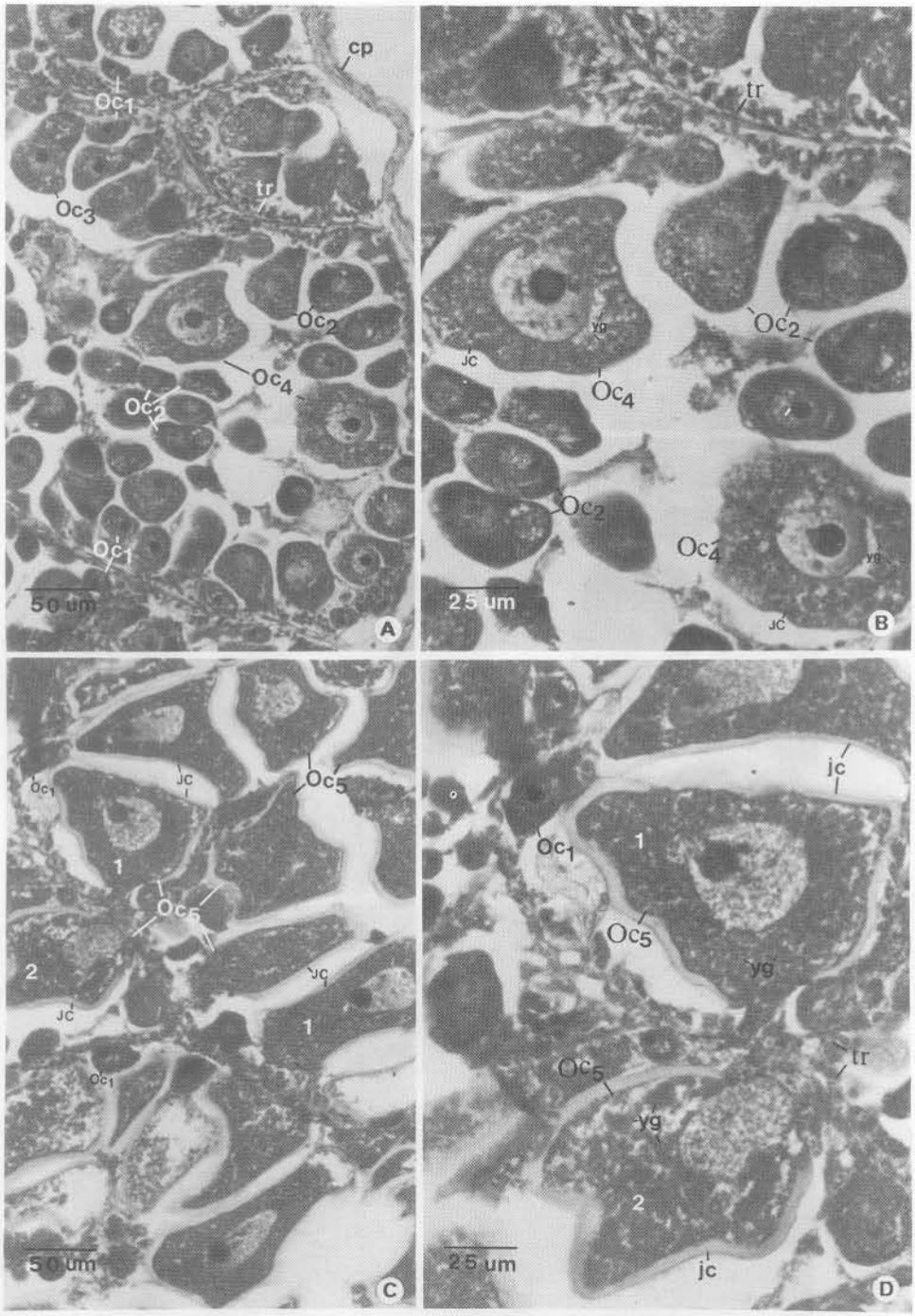


Fig.5 A-D) Paraffin sections showing stages I and II oocytes (Oc_1, Oc_2) which are abundant in A, and these cells exhibit intensely basophilic cytoplasm. In B there are two stage IV oocytes (Oc_4) showing abundant yolk platelets in the cytoplasm, and very thin jelly coat (jc). In C there are numerous stage V oocytes (Oc_5) which show intensely eosinophilic cytoplasm and thick jelly coat. In D there are two subtypes of stage V cells: the upper cell (1) shows small and evenly distributed eosinophilic yolk granules, and the lower cell (2) shows large platelets of yolk.

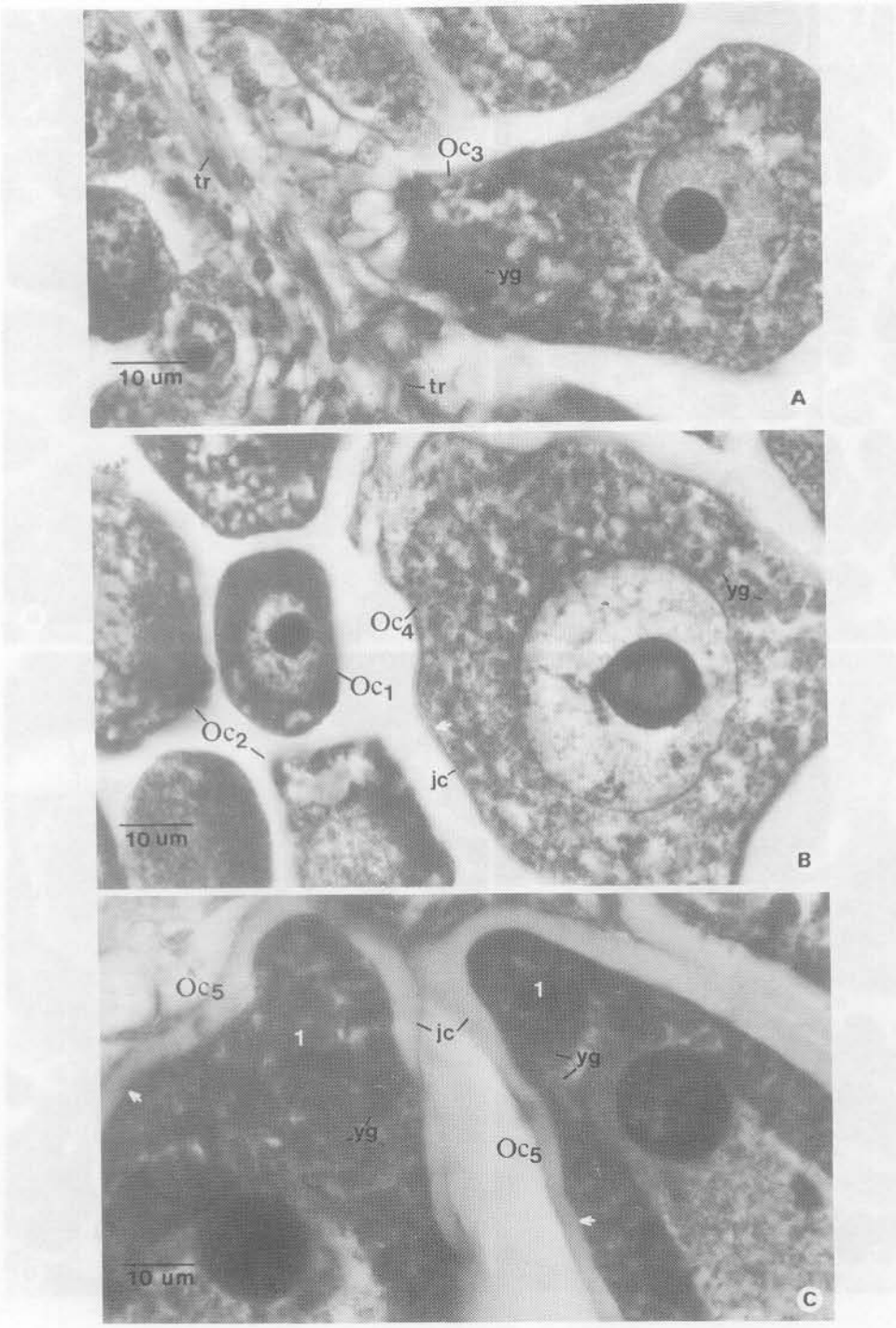
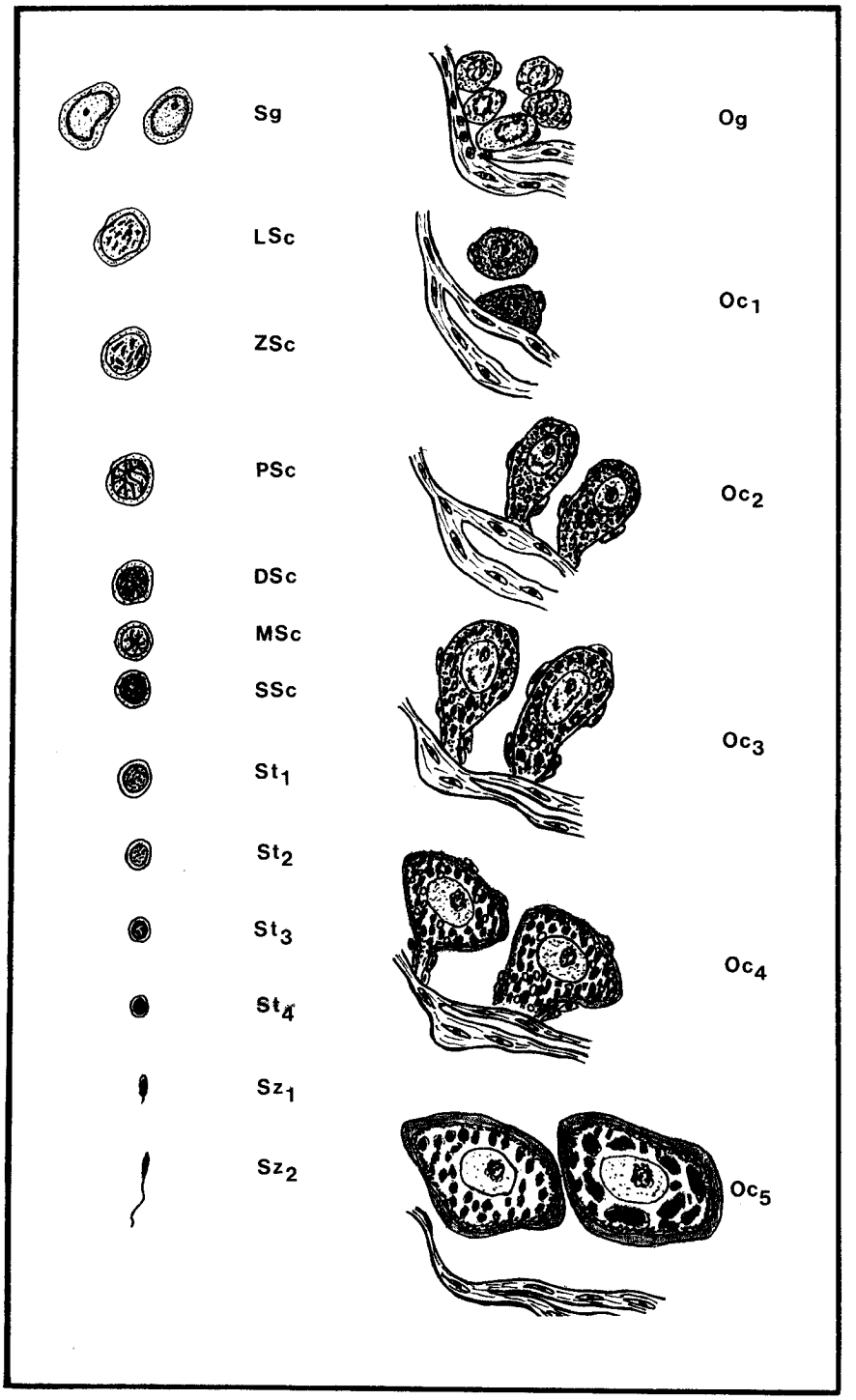


Fig.6 Paraffin sections showing stage III oocytes (Oc_3) in A, stage IV (Oc_4) in B, and stage V (Oc_5) in C. Notice the first appearance of jelly coat in stage IV, and increasing number of yolk platelets in stage IV and V. The increasing amount of euchromatin, which is pale stained, and the enlargement and vesiculation of nucleolus are also noticeable from III to V. Blue stripe underneath the oocyte's plasma membrane is present in stages IV and V.



TextFig. 1 A schematic drawing summerizes various stages of male and female germ cells with associated characteristics. In male line there are spermatogonia (Sg), five stages of primary spermatocytes (LSc-leptotene; ZSc-zygotene; PSc-pachytene; DSc-diplotene; Msc-metaphase), secondary spermatocyte (SSc), four stages of spermatids (St_{1-4}), and two stages of spermatozoa (Sz_{1-2}). In female line there are oogonia (Og), and five stages of primary oocytes (Oc_1-Oc_5).

but the remainings are translocated to concentrate as a stripe underneath the plasma membrane (Fig.5B; 6B). A thin layer of jelly coat begins to form on the outer surface of the cell membrane. This coat is PAS positive, and it is in turn surrounded by follicular cells.

Stage 5 oocyte (Oc_5): This is the fully mature oocyte before being released from the adult female. Oc_5 is the largest cells with polygonal shape (Fig.5C,D; 6C). The cell size is about 80-140 μm, with nuclear size about 40 μm. The nucleus exihibits similar characteristics as that of Oc_4, but with completely enlarged nucleolus (Fig.6C). Oc_5 could be divided into 2 subgroups based on the characteristics of yolk platelets. The first subgroup contains small and similar size yolk platelets that are scattered evenly throughout the cytoplasm. In the second subgroup, the yolk platelets are variable in size, and most are large bodies that could be formed by the coalescence of the smaller yolk platelets (Fig.5D). Stripe of fine blue granules are also located underneath the cell membrane as in Oc_4. There is a thick PAS positive jelly coat on the outer surface of the cell membrane without the surrounding layer of follicular cells. All Oc_5 are completely detached from the connectives of trabeculae.

DISCUSSION

There have been serveral studies on the gonadal histology and germ cell classification of temperate species of abalones because of the benefit that could be gained from applying the knowledge of reproductive biology, especially the reproductive cycle and spawning of mature gamete cells, to increase the yeild in aquaculture systems. Croft[7] was the first person who performed detailed morphologic studies on an abalone species, *H. tuberculata*, and showed the basic histology of the gonads being composed of fibrous capsular and trabecular arrangement, from which germ cells are generated. Similar histological studies in other species were later performed by many investigators[4-6,8-14]. A fine structural study of the ovarian cells in the red abalone, *H. rufescens*, was also undertaken by Martin *et al.*[15]. All these studies confirmed similar pattern of structural organization of the gonads. However, there are some disagreements on the classification of germ cells in the oogenetic and spermatogenic processes. Tomita[4,5] classified female germ cells into 7 stages (oogonium and 6 stages of oocytes), based on elaborate consideration of sizes and histological features; while classifying male germ cells into 4 stages (spermatogonium, spermatocytes, spermatid and spermatozoa). On the other extreme Takashima *et al.*[6] had suggested that there are up to 9 stages of female germ cells and 5 stages of male germ cells (2 stages of spermatogonia in addition to others) in *H. diversicolor diversicolor*. On the other hand, Young & DeMartini[11] suggested that there are only 4 stages of female germ cells and 4 stages of male germ cells, with ill-defined cell stages in spermiogenesis during which spermatids are transformed to spermatozoa. Utilizing the high resolution TEM to study the relative abundance of various organelles, particularly ribosomes, and the developement of rough endoplasmic reticulum and Golgi complexes in the cells, Martin *et al.*[15] suggested that there are 5 stages of female germ cells in *H. rufescens*, which they termed oogonium, presynthetic oocyte, synthetic oocyte, early postsynthetic oocyte and fully developed postsynthetic oocyte. We feel that the classification based on size alone, as adopted by many investigators, is not a good criterion for dividing cells in a single line of differentiation into various stages, because in reality these cells are undergoing continuous development. A better criterion would be to divide the cells according to the changings in histological features which exhibit definite landmarks for distinctive developmental stages. In our study of *H. asinina*, we have used the following characteristics for dividing the stages of female germ cells: (1) the appearance of chromatin and nucleolus; (2) the clarity of nuclear membrane which is the result of the density difference between the condensed chromatin in the nucleus and the surrounding cytoplasm;

(3) the basophilia or the bluishness imparted to the cytoplasm of the cells by basophilic dyes; (4) the presence of lipid droplets; (5) the occurrence of eosinophilic yolk granules and their relative abundance; and (6) the presence of jelly coat surrounding the egg cells. By using these rather stringent morphological criteria, we have identified 5 stages of egg cells, starting from oogonia which are the smallest cells closely attached to the trabecula connectives (TextFig.1). These cells could maintain a constant pool of early stem cells, particularly those that are clustered towards the capsular side of trabeculae. During the spent period when most mature oocytes are released from the ovary and the connective tissues of trabeculae are breaking down, these cells are the only remaining group of germ cells. The restoration of gonadal structure during proliferative phase is carried out by the regeneration of connective tissues of trabeculae and the proliferation of this pool of oogonia.

The first stage of oocytes include cells of different sizes ranging from 20-24 μm. The most pronounced characteristics that they exhibit is the increasing basophilia or bluishness of their cytoplasm. And because of similar degree of density between the cytoplasm on one hand, and the partially condensed chromatin and dense nucleoplasm on the other, the outline of nuclear membrane could not be easily discerned. The nucleolus, while present, is not outstanding (TextFig.1). All Oc_1 are surrounded by a single layer of flat follicular cells. We believe that the cytoplasmic basophilia reflects the increasing amount of ribosomes which will equip the cells to start synthesizing yolk proteins, much like the basophilic erythroblasts preparing themselves for synthesizing hemoglobin during the process of erythroid cell differentiation in vertebrates[16].

Oc_2 is the stage that first show the presence of lipid droplets in the less intense basophilic cytoplasm. Due to the decondensation of most chromatin, and the increased translucence of the nucleoplasm, the nuclear membrane could be clearly observed. For similar reasons the nucleolus also becomes more distinct, and because of its enlargement the nucleolar activities for ribosomal synthesis is believed to be on the increase. Contrary to the notion put foward by Martin *et al.*[15], we believe that Oc_1 and Oc_2 are actively synthetic cells, readying themselves for making yolk protein. The quiescent or presynthetic stage, if at all present, could occupy only a very short interval during the transition of oogonium to small Oc_1 cells.

Oc_3 is the stage where yolk granules, the product of synthetic activities during Oc_1 and Oc_2 stages, make their first appearance. The yolk granules or platelets are eosinophilic, hence rendering the cytoplasm of Oc_3 more reddish in contrast to that of Oc_2. We believe that this is the stage where yolk proteins start to accumulate as a result of the intense synthetic activity. Oc_3 is still surrounded by a single layer of follicular cells, which by this time consists of serveral cells because of the increase in size of Oc_3. In addition, Oc_3 is further detached from the connectives of trabeculae and assumes a pear or even tear-drop shape. The chromatin becomes completely euchromatin and the nucleolus is enlarged further, which implies the active transcriptional activities.

Oc_4 is the stage where a thin jelly coat becomes detectable, and it is sandwiched inbetween the egg's cell membrane and the surrounding layer of follicular cells. The cytoplasm of Oc_4 becomes increasingly eosinophilic and appears more reddish due to the binding of numerous yolk granules to eosin. While the jelly coat is intensely PAS positive, the yolk granules are completely PAS negative. This contrasting feature implies that there may be very little or no carbohydrate moieties in the yolk granules, while these are the major constituent of the jelly coat. The chromatin of Oc_4, like that of Oc_3, is completely in euchromatic state and the nucleolus is fully enlarged and even appears eosinophilic. These indicate very high levels of

both nuclear and nucleolar transcriptional activities.

Another remarkable feature of Oc_4 is the appearance of a narrow bluish stripe of cytoplasm just underneath the cell membrane, while the bluishness of the main body of cytoplasm is much decreased in comparison to Oc_2 and Oc_3. This could be due either to the break down of most ribosomes and the reaggregation of the residual mass into bluish clumps underneath the membrane, much like the remaining basophilic reticulum of degraded ribosomes in the cytoplasm of reticulocytes during erythroid cell differentiation[16]. Alternatively, the narrow bluish stripe could be the zone that contains a high concentration of basophilic granules, like the cortical granules, which appear in late stage of egg cells as reported in many species. Observation at electron microscopic level will help to clarify this controversy.

Oc_5 is the stage where the jelly coat becomes uniformly thick and deprived of surrounding layer of follicular cells. The cell appears completely mature and is fully detached from the trabeculae. The absence of follicular cells might allow the detachment of Oc_5 into space between trabeculae and ready to be released from the ovary. From this appearance it could be speculated that the major roles of follicular cells are protective and helping to maintain the adherence between oocytes and trabecula connective tissue, while the latter is undergoing maturation. In addition, follicular cells could be involved in nutritive function for oocytes, and its role in synthesizing the jelly coat could be envisaged by electron microscopic and labelling studies.

The cytoplasm of Oc_5 is laden with reddish yolk granules. Based on the size of the yolk granules there could be 2 subgroups of Oc_5: one containing small granules of uniform size while another contains very large granules. It is still not possible to confirm whether these are two separate stages of Oc_5, or that the latter merely represent the final stage in which small yolk granules are coalesced to form large granules. In any cases these two subgroups of Oc_5 should represent fully mature cells.

Up to now most studies have not rigorously catagorize various spermatogenic cells of *Haliotis*, apart from suggesting broadly that there are 4 stages, *ie.*, spermatogonium, spermatocytes, spermatids and spermatozoa. In our study, we could classify the male germ cells in *H. asinina* into 13 specific stages according to size, the appearance of chromatin and the presence or absence of nucleolus. Spermatogonium is the earliest cell whose nucleus contains almost all euchromatin which results in the nucleus being very clear and nucleolus is prominent. Gonial cells divided mitotically to give rise to primary spermatocytes, which pass through 5 stages as in the first meiotic division of vertebrates' germ cells (TextFig.1). These prophase cells exhibit different forms of chromatin condensation, starting from small to larger blocks of heterochromatin that are evenly scattered throughout nucleus in LSc and ZSc. Heterochromatin blocks transform to thread-like pattern that are increasing in thickness and become more entwined in PSc and DSc. Finally in MSc stage chromatin appears as pairs of chromatids that are arranged on the equatorial region. Secondary spermatocytes are rare since they probably transit quickly to the next stage similar to the cases of vertebrates' male germ cells.

Four stages of spermatids could be identified in *H. asinina*. The first two stages exhibit finely granulated chromatin that appears homogeneous and evenly stained throughout the nucleus. St_1 and St_2 is thus distinguished mainly by the difference in size (St_1 about 6 μm versus St_2 about 4 μm). In the third stage (St_3) the granulated chromatin begins to clump together, particularly along the nuclear envelope, leaving clear areas between blocks of dense chromatin. The decrease in volume of the nucleus results in the total clumping and condensing

of chromatin mass in St_4. The two stages of spermatozoa are distinguished by their ellipsoid nuclei. Sz_1 also shows the initial formation of acrosome as a clear cap-like structure on one end of the nucleus, while exhibiting only short tail. In Sz_2 the nucleus is elongated further and chromatin appears completely dense. They also exhibit long tails that point outwards from each trabecula. The application of TEM will resolve the state of chromatin condensation better, and help to confirm the above classification scheme. This work is currently in progress in our laboratory.

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บทคัดย่อ

อวัยวะสืบพันธุ์ของหอยเป๋าฮื้อที่ผลิตเซลล์สืบพันธุ์ คือ รังไข่และอัณฑะซึ่งมีถุงหุ้ม เยื่อเกี่ยวพันจากถุงหุ้มแทรกเข้าไปในรังไข่และอัณฑะเป็นแผง trabeculae ที่มีเซลล์สืบพันธุ์ขั้นต้นและขั้นปลายหุ้มอยู่รอบๆ ในรังไข่เซลล์สืบพันธุ์ประกอบด้วย 6 ระยะ คือ oogonium, primary oocytes ขั้นที่ I, II, III, IV และ V ซึ่งมีความแตกต่างกันตามปริมาณสาร basophilia ก้อนไขมันและสารไข่ (yolk) ที่เซลล์แต่ละขั้นสร้างขึ้น ในอัณฑะเซลล์สืบพันธุ์ประกอบด้วย 13 ระยะ คือ spermatogonium, primary spermatocytes 5 ระยะ, secondary spermatocyte, spermatids 4 ระยะ และ spermatozoa 2 ระยะ