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Oogenesis in *Amiantis umbonella* (Mollusca: Bivalvia) in Kuwait Bay, Kuwait

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The morphology of the cells during oogenesis in *Amiantis umbonella* (Mollusca: Bivalvia) was investigated from collections made between November 1997 and January 1999. Each stage is described and prominent features noted. A unique arrangement of auxiliary cells around the oocyte is described.

INTRODUCTION

Amiantis umbonella (Lamarck) (sub-class Heterodonta, order Veneroida, family Veneridae) occurs on the sandy/muddy intertidal zones throughout Asia where it is a source of cheap protein. The shell is ovate or ovate–triangular in shape, up to 80 mm in length, with each valve bearing three cardinal teeth. It varies in colour from white to violet with distinctive brown zigzag markings at the umbo (Carpenter et al., 1997).

Over-fishing and pollution in Kuwait Bay have depleted many intertidal bivalve species. This study has been undertaken to help establish management guidelines to maximize the annual recruitment of clam fisheries, especially for *A. umbonella*.

MATERIALS AND METHODS

Every month from November 1997 to January 1999, 30 *Amiantis umbonella* (30–35 mm shell length) were collected at low tide from the sand/mud intertidal zone of Al-Doha, Kuwait Bay (29°23′ N 47°45′ E). Most were partially exposed on the surface while others were up to 10 cm below it. In the laboratory they were maintained in circulating seawater aquaria at 24–25°C. Each specimen was measured with a Vernier micrometer, weighed, and dissected to separate the gonads from the intestine and hepatopancreas.

For light microscopy, samples were fixed in an isomolar Bouin's fixative at an osmolarity of 1300 mOsm for 24 h, washed, and preserved in 70% ethanol. They were then dehydrated through an ethanol series, cleared in xylene for 24 h, and embedded in paraffin wax. Serial sections (5–7 μm thick) were cut, stained with haematoxylin and eosin, mounted in DPX on microscope slides, and examined using a Nikon Microphoto FXA.

For electron microscopy, the gonads were cut into 4–5 pieces and fixed in 2.5% glutaraldehyde with 0.2M sodium cacodylate in seawater for 24 h. After washing three times in 0.2M sodium cacodylate (pH 7.4), the specimens were post-fixed in 1% osmium tetroxide in the same buffer at 4°C.

For transmission electron microscopy, specimens were dehydrated through an ethanol series, cleared in propylene oxide, and embedded in low-viscosity Epon (812) resin. Ultra-thin sections were stained sequentially in uranyl acetate and lead citrate for examination in JEM-1200 EXII at 80 K.

Specimens prepared for scanning electron microscopy were dehydrated in an acetone series, freeze-fractured, dried in a critical point drying apparatus using carbon dioxide as a transitional fluid, mounted on aluminium stubs, and coated in gold-palladium for examination in JEOL-SM 6300.

RESULTS

General morphology

Amiantis umbonella collected from Kuwait Bay were 18 to 35 mm long with those over 30 mm being sexually mature. The sex ratio was 1:1 with no hermaphrodites identified. The gonad of the dioecious *A. umbonella* is located in the viscera above the foot and ventral to the hepatopancreas. The sex could only be determined under the microscope after mechanical fracture of the gonad.

The ovary consists of a series of highly branched and/or lobulated clusters of acini surrounded by fibrous connective tissue and the haemocoel (Figure 1A). Each acinus is surrounded by a thin acinal wall and contains developing oocytes during oogenesis (Figure 1B). In some sections acini appear interconnected with each other and empty into small gonadal ducts (Figure 1C,D). The latter form a common gonoduct that opens into the epibranchial chamber where presumably fertilization takes place.

The developing oocytes in close contact with the acinal wall are the germ cells or protogonia, oogonia, previtellogenic cells and the postvitellogenic cells. All mature oocytes or ova are found in the acinal lumen.

Oogenesis

The stages of oogenesis in *Amiantis umbonella* are shown in Figure 2. They commence in the premeiotic phase and include the protogonia and oogonia stages.

The protogonial cells are round or elongate (5.5–6.5 μm in diameter) and appear in close contact with each other by means of a desmosome-like junction. The nucleus is

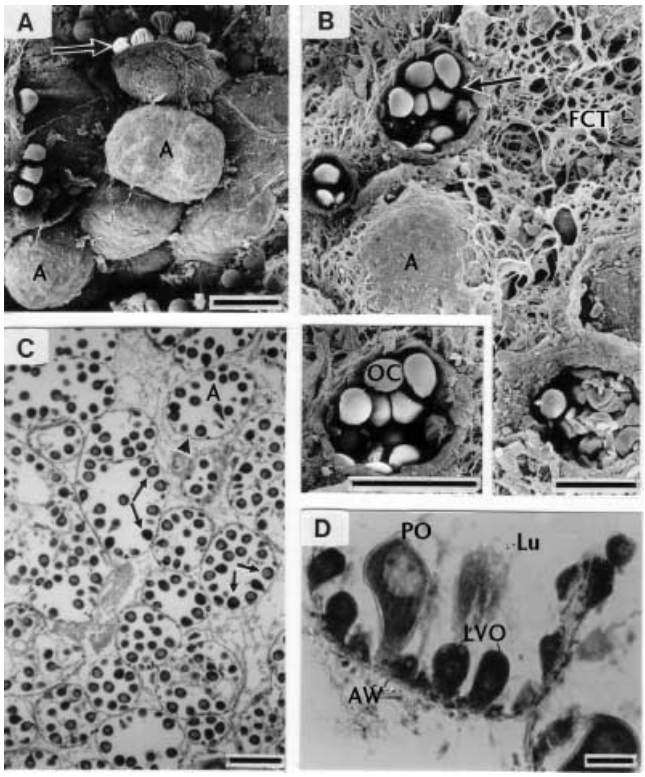

Figure 1. (A) Scanning electron microscopy (SEM) of freeze fractured portion of a female gonad showing numerous acini (A) that have a lobulated outline, some of which are ruptured to exhibit developing oocytes (arrow); (B) SEM of transverse fractured portion of a female gonad showing the circular-shaped acini with a number of developing oocytes (arrow). The acini are embedded in a network of fibrous connective tissue (FCT). Insert shows high magnification of one acinus containing a number of developing oocytes (OC); (C) light-microscopy (LM) of cross section through the active ovary of a female showing circular and semi-circular shaped acini (A) containing oocytes (arrows) at various phases of oogenesis. The gonadal acini are surrounded by a thin acinal wall (arrow-head). The photograph suggests that the acini are interconnected with each other and/or empty into a gonadal duct; (D) LM of transverse section of a female gonad of *Amiantis umbonella* showing lobulate acini. Note the various stages of developing oocytes attached to the acinal wall (AW). Scale bars: A–C, 100 μm; D, 10 μm.

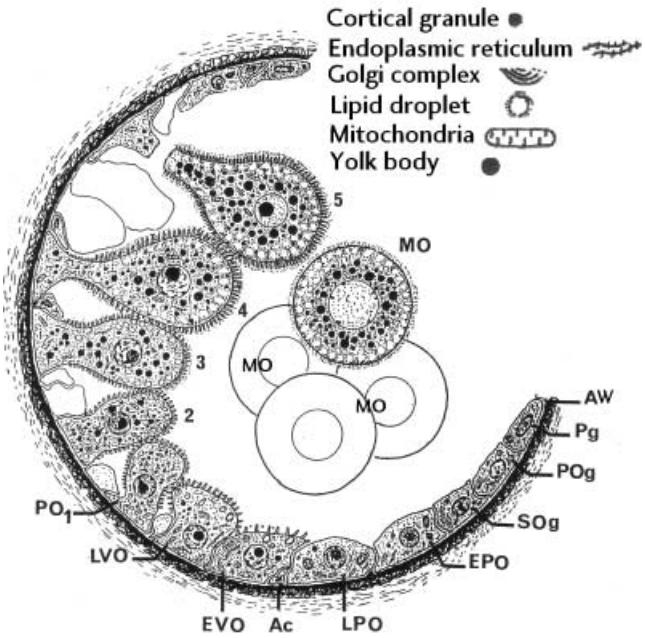

Figure 2. Diagrammatic representation of oogenesis in the clam *Amiantis umbonella* ovary, based on light and electron-micrograph observations. The various stages of oogenesis are: protogonia (Pg), primary oogonia (POg), secondary oogonia (SOg), early previtellogenic oocyte (EPO), late previtellogenic oocyte (LPO), early vitellogenic oocyte (EVO), late vitellogenic oocyte (LVO), postvitellogenic oocytes (PO_{1-5}), and mature oocytes (MO). The acinal wall (AW) holds all stages except for the mature oocyte. Some auxiliary cells (Ac) are found in association with the developing oocytes.

round or elongate with a single nucleolus (1.0 μm in diameter) containing reticular and marginal chromatin and surrounded by rough endoplasmic reticulum (RER) (Figure 3A,C).

The protogonia presumably divide mitotically to form oogonia on the inner wall of the acinus. The oogonia stain less intensely with toluidine-blue than do the protogonia and occur in one or two layered cells forming two substages namely primary and secondary oogonia (Figure 3A,B). Primary oogonia are irregular in shape, measure 6.0–7.0 μm in length with a large nucleus (2.5 μm in diameter). The cytoplasm contains few mitochondria, ribosomes, and endoplasmic reticular cisternae. Secondary oogonia are elongate (6.0–7.5 μm) having an ovoid nucleus (2.5×4.0 μm) with no visible nucleolus (Figure 3B). Numerous large mitochondria, Golgi complexes, and some endoplasmic reticular cisternae are found in the cytoplasm. The oogonia are attached to each other by desmosomes (Figure 3C) and usually lack microvilli.

The secondary oogonia form large, round previtellogenic cells with large nuclei containing a single nucleolus. Chromatin material is restricted to around the periphery of the nucleus. During this phase a few small auxiliary cells occur associated with the acinal wall (Figure 4A). The large cytoplasm of previtellogenic cells has few organelles while the nucleus contains small dense masses associated with chromosomal changes during diplotene (Figure 4B,C).

Early previtellogenic oocytes are ovoid (11.0–15.0 μm long) with numerous mitochondria and RER cisternae in the cytoplasm (Figure 4B). Auxiliary cells are seen in close contact with the oocyte with no desmosome. The late previtellogenic oocytes are large and elongate (17–20 μm long) with heterochromatin scattered throughout the nucleoplasm with a prominent, eccentric nucleolus.

In the vitellogenic phase the oocytes have a large central nucleus (10.0–13.0 μm in diameter) and a single nucleolus attached to the nucleolemma. Cells re-orientate as they enlarge into the lumen while attached to the acinal wall only by their base. Auxiliary cells are now limited to the basal region of the oocyte (Figure 5A). The vitellogenic oocytes develop an extensive microvilli brush border with a vitelline coat between the microvilli (Figure 5B,C). In the early stages electron-dense granules are found near the Golgi complex that may be the precursors of the yolk. The endoplasmic reticulum cisternae occur around the yolk bodies and the mitochondria. Some lipid droplets occur in the periphery of the oocyte

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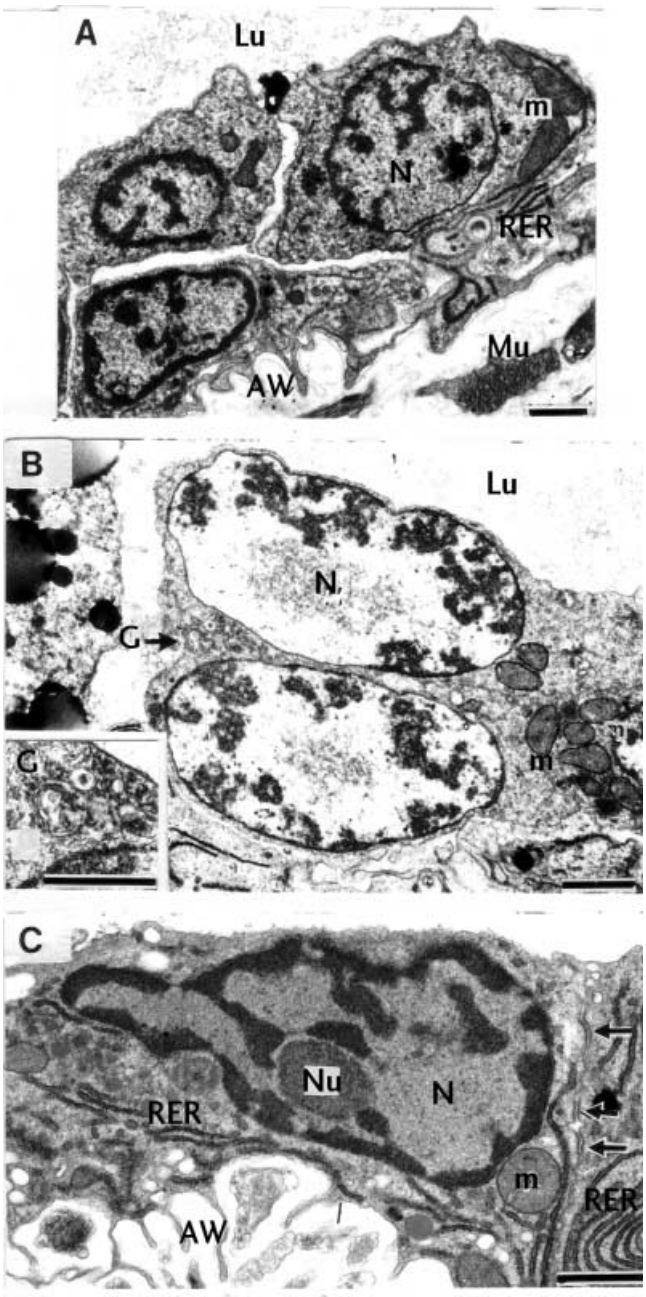

Figure 3. Transmission electron micrographs showing protogonia and oogonia during oogenesis in *Amiantis umbonella*. (A) Acinal cells of premeitotic phase oogonia. Primary oogonia are attached to the inner side of the acinal wall (AW). They have large nucleus (N) with few chromatin material. The cytoplasm has few mitochondria (m). Muscle fibres (Mu) are seen in the connective tissue; (B) secondary oogonia are in final phase of mitotic division showing large, elongate nucleus (N). Mitochondia (m) and Golgi complex (G) are seen in the cytoplasm. Insert: high magnification of Golgi body (G); (C) protogonial cell with elongate nucleus (N) and a single nucleoulus (Nu) attached with other protogonia by three desmosomes (arrows). Mitochondria (m) are found in the cytoplasm. The cell is lying on the inner face of the acinal wall (AW). Scale bars: A–C, 1 μm.

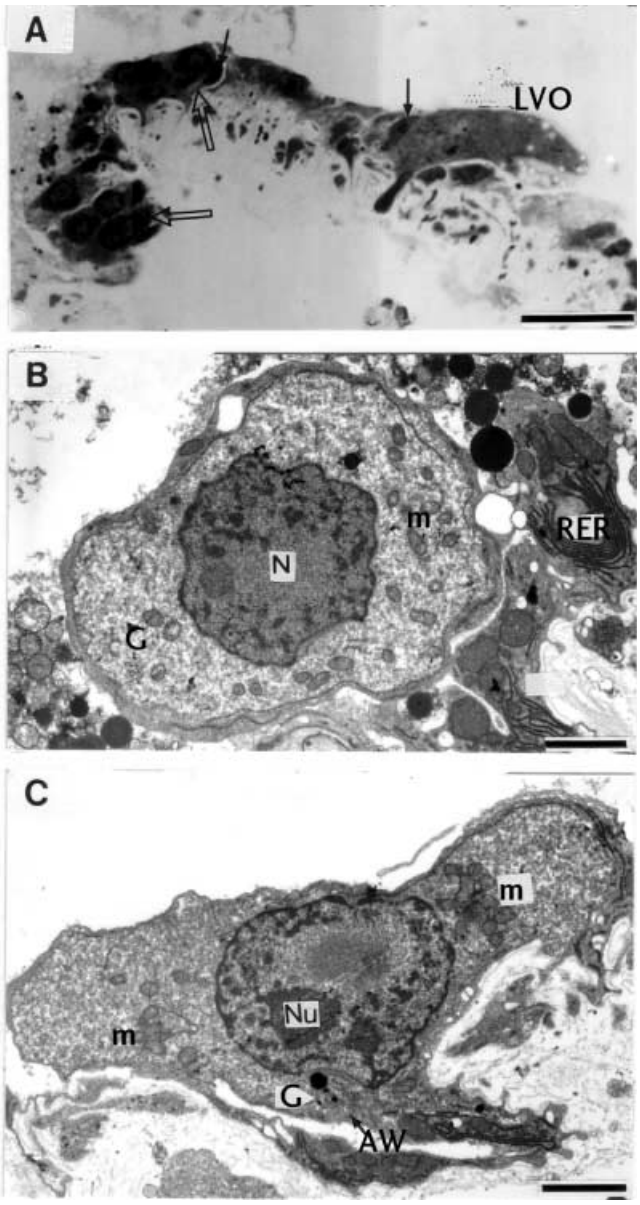

Figure 4. Light microscopy (LM) and transmission electron microscopy (TEM) showing previtellogenic oocytes. (A) LM showing early previtellogenic oocytes (open arrows) and late vitellogenic oocytes (LVO) on acinal wall in association with small auxiliary cells (black arrows); (B) TEM of early previtellogenic oocyte showing central nucleus (N), few scattered mitochondria (m) in the cytoplasm, and the Golgi complex (G). The rough endoplasmic reticulum (RER) of the nearby auxiliary cell can be seen; (C) TEM of late stage previtellogenic oocyte showing eccentric nucleolus (Nu) within the nucleus (N). Numerous mitochondria aggregate close to nuclear membrane. Golgi complex (G) and cisternae of endoplasmic reticulm (ER) are seen in the cytoplasm. Note the large surface area of the cell in contact with the acinal wall. Scale bars: A, 15 μm; B&C, 2 μm.

often surrounded by glycogen granules (Figure 5D). The nucleus is irregular in shape having an eccentric nucleolus (4.5 μm in diameter). Few auxiliary cells with prominent oblong nuclei with scattered heterochromatin are restricted to the basal region of the vitellogenic oocytes lacking microvilli.

During vitellogenesis the vitelline coat becomes large and clear, and the RER forms whorls and/or concentric arrays of cisternae around the mitochondria or yolk bodies (Figure 6A–C). The yolk bodies are spherical,

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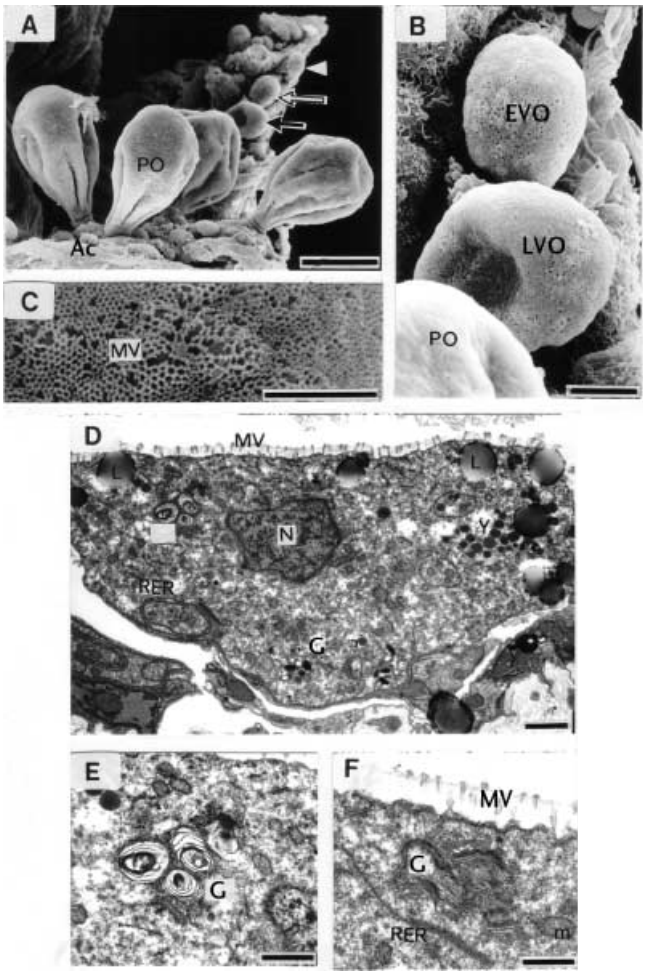

Figure 5. Scanning electron (SEM) and transmission electron (TEM) micrographs showing early vitellogenesis. (A) SEM of portion of active female gonad to show different stages of vitellogenesis starting from previtellogenic oocyte (arrowhead), early vitellogenic oocyte (white arrow), late vitellogenic oocyte (black arrow), and postvitellogenic oocyte (PO); (B) SEM of early vitellogenic oocyte (EVO) with late vitellogenic oocytes (LVO). Note the thick microvilleous brush border (MV) that covers the oocyte surface; (C) high magnification of microvilli border; (D) TEM of early vitellogenic oocyte showing microvilli (MV) and proliferation of intracellular organelles such as Golgi complex (G), moderately well developed whorls of rough endoplasmic reticulum (RER). Lipid droplets (L) occur on the periphery of the cell with few yolk bodies and mitochondria (m) scattered in the cytoplasm. The nucleus is irregular in shape; (E) high magnification of D to show Golgi bodies (G); (F) high magnification showing active Golgi complex (G) and strands of endoplasmic reticulum (RER). Note the pinocytic depressions and the deposits of material at the apical plasmalemma between the microvilli (MV) brush border. Scale bars: A, 50 μm; B, 10 μm; C, 5 μm; D&F, 2 μm; E, 1 μm.

electron-dense, and membrane-bound granules (0.5–1.5 μm in diameter). Pinocytosis occurs at the apical region of the cells (Figure 5D,E) and cortical granules are seen with an undulating membrane. The cell changes to form a conspicuous stalk or peduncle attaching it to the acinal wall.

In the postvitellogenic phase the large oocytes are polyhedral and compressed tightly together within the mature

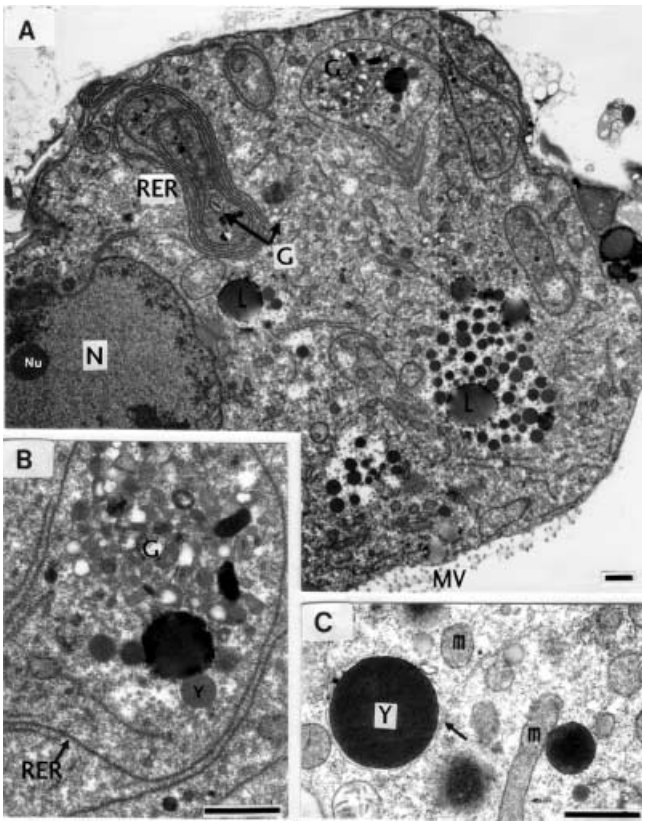

Figure 6. Transmission electron microscopy of late vitellogenic oocytes. (A) Late vitellogenic oocyte showing proliferation of lipid droplets (L), yolk granules (Y), whorls of RER, as well as the nucleus (N), the nucleolus (Nu), and the Golgi complex (G); (B) high magnification of the yolk (Y) synthesis in the vicinity of the RER whorls and the Golgi complex (G); (C) high magnification of yolk body (Y) to show close association with flattened cisternae of endoplasmic reticulum (RER). Immature yolk granules of small to medium size are also seen in the cytoplasm as are elongate mitochondria (m). Scale bars: A–C, 1 μm.

gonad (Figure 7A–D). The cell nuclei are large and spherical (20–35 μm in diameter) with either one nucleolus (10 μm in diameter) or two smaller nucleoli (4 μm in diameter) attached to the nucleolemma (Figure 7D).

Early postvitellogenic oocytes appear to bulge into the centre of the acinal lumen while the basal stalk attaches them to the acinal wall (Figure 5A). Later the oocytes detach from the acinal wall, reabsorb the stalk to become spherical (65–75 μm in diameter), and have a spherical nucleus and a nucleolus (Figure 8A,C).

During the postvitellogenic phase lipid droplets in the cytoplasm increase in size and become concentrated at the periphery of the cell plasmalemma (Figure 8A,D). Likewise the yolk bodies become membrane bound with the high electron dense homogenous cortex. Cortical granules with electron-dense matrices are found surrounded by an irregular membrane (Figure 8D,E). Numerous vesicles with a translucent matrix are observed bordering the base of the micovilli and may be involved in the separation of the vitelline coat from the surface of the oocyte to form the thin perivitelline space (Figure 8D).

Mature oocytes aggregate in the centre of the acinal lumen in the mature ovary (Figure 1B). They are spherical (65–75 μm in diameter) and initially retain their basal

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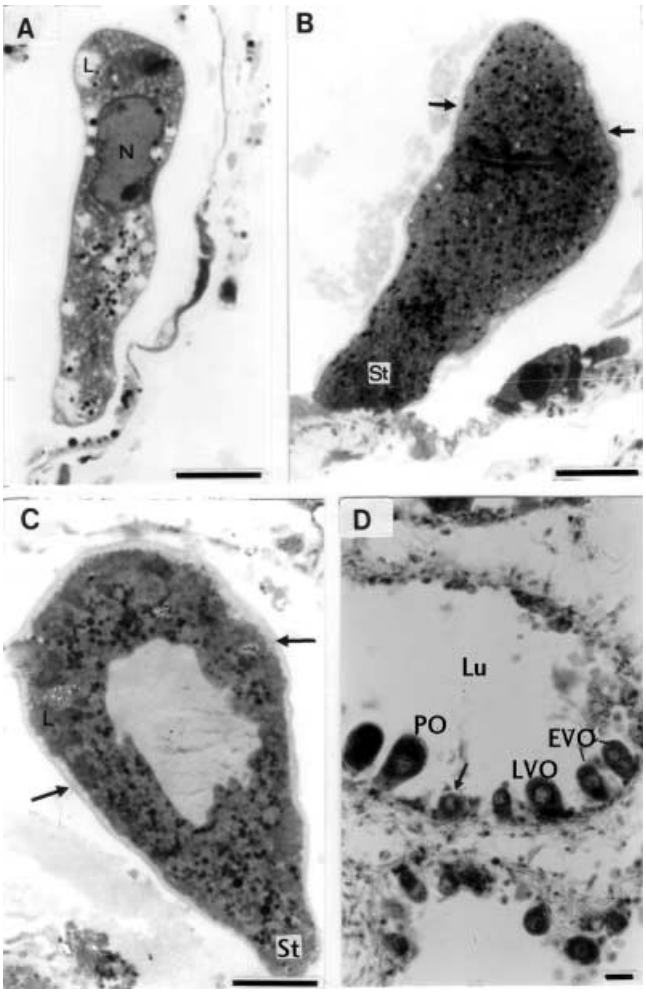

Figure 7. Light micrographs showing postvitellogenic oocytes. (A) Early postvitellogenic oocyte showing elongate-shaped nucleus (N) and scattered lipid droplets (L) in the cytoplasm. Formation of yolk is well developed (black dots); (B) mid-postvitellogenic oocyte with large amounts of lipid droplets (L) and still attached to the acinal wall by the stalk (St). The vitelline membrane (arrows) is well established; (C) a postvitellogenic oocyte showing the distal bulge into the lumen of the acinus with narrow stalk (St) and crenulated nucleus (N); (D) transverse section of active ovary showing previtellogenesis (*), early vitellogenesis (EVO), late vitellogenesis (LVO), and postvitellogenesis (PO). Scale bars: A–D, 20 μm.

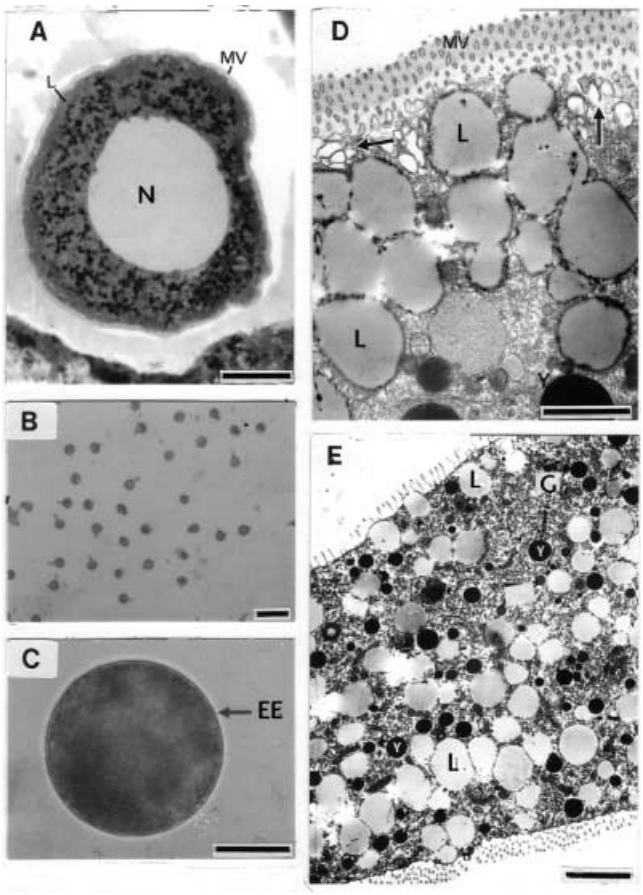

Figure 8. Mature oocytes of clam, *Amiantis umbonella*. (A) A detached mature oocyte in the lumen of the acinus. There are large numbers of spherical lipid droplets (L) at the periphery and a well-developed vitelline membrane (VM); (B) light microscopy showing release of mature eggs during spawning; (C) ripe ova with well-developed egg envelope (EE); (D) transmission electron microscopy (TEM) of mid-postvitellogenic oocyte showing concentrations of lipid droplets (L) beneath the microvilli (MV). Note the perivisceral space (arrow head) beneath the microvilli; (E) TEM of mid-postvitellogenic oocyte showing prominent lipid drolets (L) in the ooplasm. Cortical granules (CG), Golgi complex (G), and yolk bodies (Y) are abundant in the cytoplasm. Scale bars: A, 20 μm; B, 180 μm; C, 25 μm; D&E, 2 μm.

stalk that is slowly reabsorbed as the egg envelope is formed and the oocytes mature. The oocyte is surrounded by an oocyte envelope, contains lipid droplets (1.5–2.5 μm in diameter), yolk bodies (0.5–1.5 μm in diameter), and cortical granules. The cytoplasm contains numerous mitochondria and vesicular endoplasmic reticulum with most cytoplasm near the nucleus (Figure 9A,B). Often a fibrous intermicrovillar matrix 2.5 μm thick is seen along the microvilli (Figure 9C).

The *Amiantis* egg is 65–75 μm in diameter at shedding and one female may produce 12×10^3 eggs per spawning period. After shedding, the oocytes degeneration takes place when the remaining postvitellogenic oocytes are reabsorbed after a lytic material is released into the acinal lumen (Figure 9D).

DISCUSSION

The sequential changes in ultrastructural morphology of *Amiantis umbonella* during oogenesis are reported for the first time. The oocytes develop within acini that make up the female gonad. Each acinus is surrounded by connective tissue with haemocoelic sinuses and intermittent myoepithelial cells. The general features of the *Amiantis* gonad are similar to most bivalves such as *Mytilus edulis* (Pipe, 1987a), *Pecten maximus* (Dorange & Le Pennec, 1989a), *Pinna nobilis* (Gaulejac et al., 1995a), and *Crassostrea virginica* (Eckelbarger & Davis, 1996a).

Protogonia occur in groups of five to six cells in *Pinna nobilis* and contain lipid droplets (Gaulejac et al., 1995). In *A. umbonella* no such groupings and no lipid droplets were seen in this stage. The mitotic figures in the protogonia of *A. umbonella* are indicative of the cells entering mitosis as reported by Gaulejac et al. (1995a).

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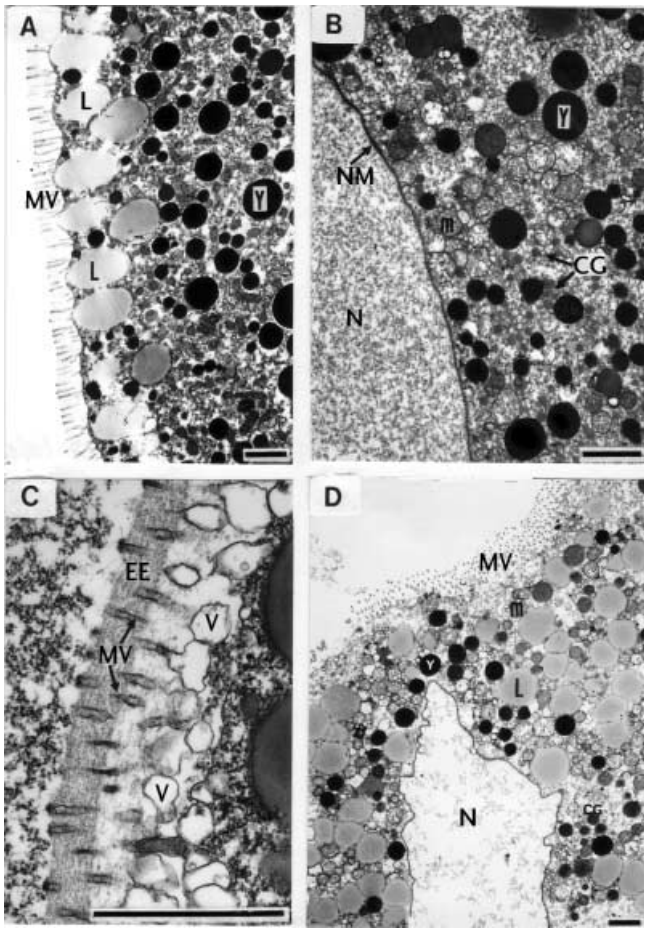

Figure 9. Transmission electron microscopy of mature oocytes. (A) Portion of mature oocyte showing uniform layer of lipid droplets (L) and scattered yolk bodies (Y). It is surrounded by a well-developed microvilli (MV) border; (B) portion of mature oocyte showing part of nucleus (N) surrounded by a nuclear membrane (NM). The ooplasm contains large numbers of cortical granules (CG), mitochondria (m), and yolk bodies (Y); (C) high magnification of the egg envelope (EE) to show microvilli (MV) and electron-lucent vesicles (V); (D) a degenerated oocyte with irregular-shaped nucleus (N) showing micovilli (MV) and releasing oocytic organelles that include cortical granules (CG), lipid droplets (L), mitochondria (m), and yolk bodies (Y) into the lumen of the acinus. Scale bars: A–D, 2 μm.

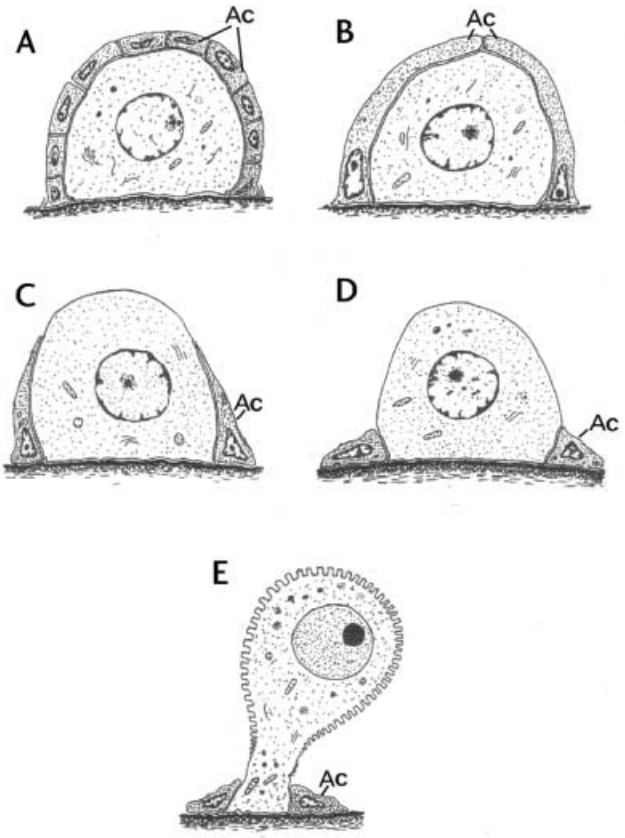

Figure 10. Diagrammatic illustration of auxiliary cell types found in the bivalves: (A) Type 1. Cells completely surround the oocytes (Jong-Brink et al., 1983); (B) Type 2. Cells mostly surround the oocytes (Jong-Brink et al., 1983); (C) Type 3. Cells partially surround the oocytes (Pipe, 1987); (D & E) Type 4. Cells are always restricted to the base of oocytes (present study).

The changes from primary oogonia to secondary oogonia that enter the first meiotic division to become oocytes are reported for *Mytilus edulis* (Pipe, 1987a) and for *Pecten maximus* (Dorange & Le Pennec, 1989a). Gaulejac et al. (1995a) describe oogonia cell division as the zygotene–pachytene stage of meiosis because of the presence of synaptonemal complexes in the nucleus. In contrast Eckelbarger & Davis (1996a) reported no distinct population of mitotic oogonia in *Crassostrea virginica*. In *A. umbonella* the images of mitotic figures demonstrate cellular division of the oogonia. The premeiotic phase cells (protogonia and oogonia) may be responsible for the multiplication of acinal oocytes that Anderson (1974) described as being the generative/proliferation stage of oogenesis.

The previtellogenic oocytes show no mitotic activity having clear nuclei and nucleoli while the large cytoplasm is indicative of the onset of cellular growth. The ultrastructure of previtellogenic and vitellogenic oocytes in *Amiantis* follows the pattern found in other bivalves, *Pecten maximus* (Dorange & Le Pennec, 1989a), *Pinna nobilis* (Gaulejac et al., 1995a), and in *Crassostrea virginica* (Eckelbarger & Davis, 1996a). One clear difference is that the above authors all reported lipid droplets and granule inclusions in previtellogenic cells that were not seen in *Amiantis*.

In *Amiantis* the vitellogenic oocyte is a growth phase involving morphological modification, proliferation of organelles and active synthesis of interacellular inclusions. Early stage elongate cells of 24.5 μm in diameter enlarge to become a bulging tear-shaped late stage of 34.5 μm. The presence of microvilli on the cell surface is an important feature for increasing surface area and absorption of macromolecules from the acinal lumen. The numerous Golgi complexes with arrays and annulated RER cisternae are indicative of cellular synthetic activity, as are the progressive multiplication and accumulation of yolk bodies, lipid droplets, and cortical granules. Similar observations have been reported for other bivalves; *Mytilus edulis* (Pipe, 1987a), *Hinnites giganteus* (Malachowski, 1988), *Pecten maximus* (Dorange & Le Pennec, 1989a), *Pinna nobilis* (Gaulejac et al., 1995a), *Crassostrea virginica* (Eckelbarger & Davis, 1996a), *Pinctada fucata* (Behzadi et al., 1997).

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The literature is not clear as to the precursors of yolk and yolk synthesis in bivalves. In *Pecten maximus* two types of vitelline (yolk) inclusions of a lipid and proteinaceous nature have been described (Dorange & Le Pennec, 1989a). In *Pinnia nobilis* Gaulejac et al. (1995a) reported two types of vitelline (yolk) inclusions, a proteinaceous yolk granule produced by the Golgi complexes from smaller granules and the other a lipid-proteinaceous yolk granule formed from lipid globules and dilated cisternae of RER.

Eckelbarger & Davis (1996a) postulated that in *Crassostrea virginica* endocytotic activity along the plasmalemma in the basal region of the vitellic oocytes are where large macromolecules of the yolk are heterosynthetically incorporated into the oocytes from the haemocoel. They suggested that the process of autosynthesis of yolk bodies involved the combined activity of Golgi complex and RER; the heterosynthesis of yolk bodies involves extraovarian precursors incorporated into the oocytes via endocytosis. While an immunocytochemical study of vitellogenesis in *Crassostrea gigas* found a female-specific protein from the haemolymph and a related ovarian protein localized in the ovary (Suzuki et al., 1992).

In *Amiantis* the morphology suggests that the RER cisternae, Golgi complex and dense vesicles are sequentially involved in yolk body production by autosynthesis to form yolk cells inside the vitellogenic oocytes. The presence of microvilli and several pinocytic invaginations along the free plasmolemma suggest the uptake of exogenous materials from the acinal lumen that may include yolk precursors. The lipid droplets in *A. umbonella* late vitellogenic oocytes act both as an energy store and to increase buoyancy in fertilized eggs (Gabbott, 1975). The role of the cortical granules in vitellogenic oocytes is undetermined. In *A. umbonella* the vitellogenic and postvitellogenic phases form the major period of oogenesis. During this time three biosynthetic mechanisms are encountered to produce the yolk bodies, lipid droplets, and cortical granules. These will eventually be used by the mature oocytes in post-fertilization embryonic development.

The auxiliary cells are probably derived from unequal division of germ cells during mitosis. Eckelbarger & Davis (1996a) suggest that in *Crassostrea virginica* the follicle 'auxiliary' cells may have a nutritive role and may regulate the flow of metabolites into the oocytes. In *Amiantis* the role of microvilli and auxiliary cells are morphologically revealed and seem to have an important role during oocyte development. The position of the auxiliary cells between the oocytes and the acinal wall may regulate the flow of small ions and micromolecules from the haemolymph. These materials may be reassembled into metabolites by the elaborate RER and transferred via the desmosome-like junctions to the oocytes. The role of the extensive microvilli and the presence of pinocytosis are indicative of absorption of metabolites from the acinal lumen. The transport of materials into the oocytes needs further investigation using cytochemical techniques.

Interestingly there are three types of auxiliary cell–oocyte relationship in bivalves reported in the literature. They are: Type 1 where the auxiliary cells completely surround the oocyte; Type 2 where the auxiliary cells mostly surround the oocyte but not completely; and Type 3 where the young oocycte is completely surrounded by the auxiliary cells that during oogensis become restricted to the basal region of the oocyte. Jong-Brink et al. (1983) report the first two Types while Pipe (1987) reports Type 3 for *Mytilus edulis*. In *Amiantis umbonella* there exists a Type 4 where the auxiliary cells only occur around the basal region of the oocyte at all stages of development (Figure 10). The effects of the different arrangements of auxiliary cells and the presence of microvilli may affect the transport of metabolites during oogenesis. This is under investigation in *Amiantis* by the authors.

After shedding, the remaining postvitellogenic oocytes undergo degeneration involving the breakdown of the plasma membrane and the components are reabsorbed. This phenomenon has been reported in other molluscs (Pipe, 1987a; Dorange & Le Pennec, 1989a; Gaulejac et al., 1995a; Eckelbarger & Davis, 1996a). The timing and mechanisms of this process are not clear and need further investigation.

Reproduction in *Amiantis* may be controlled by temperature changes as suggested by Sastry (1979) and Behzadi et al. (1997). During the very hot summer up to September when the water temperatures are $>32.7^{\circ}$C the sexual development in *A. umbonella* in Kuwait Bay appears restrained. As temperatures decline in late October and early November to around 24°C, sexual development resumes.

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REFERENCES

Anderson, E., 1974. Comparative aspects of the ultrastructure of the female gamete. *International Review of Cytology*, **4**, 1–70.

Behzadi, S., Parivar, K. & Roustaian, P., 1997. Gonadal cycle of pearl oyster, *Pinctada fucata* (Gould) in northeast Persian Gulf, Iran. *Journal of Shellfish Research*, **16**, 129–135.

Carpenter, E.K., Krupp, F., Jones, D.A. & Zajonz, U., 1997. *Living marine resources of Kuwait, eastern Saudi Arabia, Qatar, and the United Arab Emirates*. Rome: Food and Agricultural Organization of the United Nations.

Dorange, G. & Le Pennec, M., 1989. Ultrastructural study of oogenesis and oocytic degeneration in *Pecten maximus* from the Bay of St. Brieuc. *Marine Biology*, **103**, 339–348.

Eckelbarger, K.J. & Davis, C.V., 1996. Ultrastructure of the gonad and gametogenesis in the eastern oyster *Crassostrea virginica*. I. Ovary and oogenesis. *Marine Biology*, **127**, 79–87.

Gabbott, P.A., 1975. Storage cycles in marine bivalve molluscs: a hypothesis concerning the relationship between glycogen metabolism and gametogenesis. In *Proceedings of the 9th European Marine Biology Symposium* (ed. H. Barnes), pp. 191–211. Aberdeen: Aberdeen University Press.

Gaulejac, J., Henry, M. & Vicente, N., 1995. An ultrastructural study of gametogenesis of the marine bivalve *Pinna nobilis* (Linnaeus 1758). I. Oogenesis. *Journal of Molluscan Studies*, **61**, 372–392.

Jong-Brink, M. de, Boer, H.H. & Joose, J., 1983. Mollusca. In *Reproductive biology of invertebrates*, vol. 1 (ed. K.G. Adiyodi and R.D. Adiyodi), pp. 297–335. Chichester: John Wiley.

Malachowski, M., 1988. The reproductive cycle of the rock scallop *Hinnites giganteus* (Grey) in Humboldt Bay, California. *Journal of Shellfish Research*, **7**, 341–348.

Pipe, R.K., 1987. Oogenesis in marine mussel *Mytilus edulis*: an ultrastructural study. *Marine Biology*, **95**, 405–414.

Sastry, A.N., 1979. Pelecypoda (excluding Ostreidae). In *Reproduction of marine invertebrates*, vol. V. *Molluscs: pelycypods and lesser classes* (ed. A.C. Giese and J.S. Pearse), pp. 113–292. New York: Academic Press.

Suzuki, I., Hara, A., Yamaguchi, K. & Mori, K., 1992. Purification and immunolocation of a vitellin-like protein from the Pacific oyster *Crassostrea gigas*. *Marine Biology*, **113**, 239–245.

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