The Zoologist Vol. 4, 18-26 (2006)

AN ULTRASTRUCTURAL STUDY OF OOGENESIS IN *ARCHACHATINA MARGINATA OVUM* (PFEIFFER) (PULMONATA: ACHATINIDAE).

ROSEMARY I. EGONMWAN
Department of Zoology, University of Lagos, Akoka, Lagos, Nigeria.

Abstract

Morphology of the cells during oogenesis was studied in the ovotestis of the edible land snail *Archachatina marginata ovum* (Pfeiffer) using light and transmission electron microscopy. The ovotestis is an acinus organ containing sperm and oocytes in various stages of gametogenesis. Most acini contain only spermatogenic cells and in only a few are oocytes and spermatozoa found developing and maturing together. Four stages of oogenesis were identified and described; these are premeiotic protogonia, oogonia, previtellogenic oocytes and vitellogenic oocytes. Previtellogenic oocytes contain few organelles and are surrounded by follicle cells. Three types of storage products were identified in cytoplasm of the oocytes during vitellogenesis; these are yolk, lipid and glycogen.

Key words: oogenesis, *Archachatina marginata ovum*, oocytes, follicle cells, yolk granules.

Introduction

Archachatina marginata ovum (Pfeiffer, 1858) is an edible snail which belongs to the family Achatinidae. *Archachatina (Calachatina) marginata* (Swainson, 1821), the giant African land snail occurs mostly in coastal regions, from Republic of Benin in West Africa to Zaire in Central Africa (Bequeart 1950; Mead, 1950). The giant land snails are economically very important in many West African countries as a cheap protein source (Ajayi *et al.*, 1978).

Like all pulmonates, achatinids are hermaphrodites with internal fertilization; majority of them are either simultaneous hermaphrodite (Heller, 1993) or protandric hermaphrodite (Pal and Hodgson, 2005). Although this snail has been studied extensively, many aspects of their reproductive biology are still unknown. Odiete (1981 & 1982) gave a summary of the fine structure of gametes from the ovotestis of *A. marginata ovum*. However, the studies did not show the relationship among the different gametogenetic cells and the author admitted that the account of the gametogenesis in this snail was far from complete.

Oocyte differentiation and maturation comprises a number of processes of which formation of yolk granules during vitellogenesis is very important (Jong-Brink and Garaerts, 1982) and in most molluscs, the process is mostly by autosynthesis in the perinuclear cytoplasm at the beginning of vitellogenesis (Medina *et al.*, 1986; Pal and Hodgson, 2002). In the prosobranch gastropod *Bathynerita*, yolk formation by the incorporation of exogenous yolk precursors by endocytosis during mid to late vitellogenesis has also been reported (Eckelbarger and Young, 1997). The amount of yolk accumulated in oocytes and hence the size of the oocytes, varies greatly among molluscs (Pal and Hodgson, 2002) and depends on if the species has a direct or planktonic larval development (Pal and Hodgson, 2005). The yolk contained in the eggs is the main source of nutrition for the embryo of cephalopods, but in most gastropods, the oocytes contain moderate amount of yolk and perivitelline fluid secreted by the albumen gland. The eggs develop directly into a juvenile after about 2 weeks (Egonmwan, 2004). *A. marginata ovum* produces few clutches of 4-16 very

*Tel. +234 (1) 545-4891: Fax: +234 (1) 493-2669;
e-mail: egone@infoweb.com.ng

large lemon-yellow eggs with hard calcareous shell, about 0.82% of parent's body weight. The eggs are laid beneath soil and leaf litters and are coated with a thin film of mucus that protects them from drying and rolling (Egonmwan, 2004).

In recent years more attention has been paid to the commercial farming of Achatinid land snails in many West African countries as opposed to the exploitation of natural population which has been greatly depleted by deforestation, bush-burning and over-exploitation because of the large size of the snail which makes it a great commercial potential. Knowledge of reproductive biology is central to management of gastropod populations for conservation, control of pest species in agriculture, control of helminth parasite transmission or captive rearing of edible species (Gomez, 2001).

The aim of this study, therefore, was to investigate and describe the pattern of oogenesis in *A. marginata ovum*. In addition, the fine structure of oogenesis is described using microscopic techniques.

Materials And Methods

Maintenance of snails in the laboratory

A colony of *A. marginata ovum* obtained from a site at Iguobazuwa village, near Benin City in the rainforest area of southwest Nigeria (lat. 06° 19¹ N: long. 05° 36¹ E) was maintained in constant temperature room where the thermostat was set at 25.0 °C and the relative humidity of the room was about 60-70%. The snails were reared in glass tanks and plastic bowls which had layers of moist humus soil at the bottom. The snails were fed twice a week *ad libitum* on a mixture of lettuce, potato, carrot, apple and pawpaw leaves. Uneaten food was removed before fresh one was provided.

Sample preparation

The snail specimens used for this study were obtained from the laboratory culture, so were of known age. Specimens from the wild were also examined for comparison. A small sample of two to three snails was removed from the culture each month, and each snail was weighed on a Mettler 100 AE electronic balance. The juveniles were measured under a Zeiss stereomicroscope equipped with an ocular micrometer, and the adults were measured with a vernier caliper. The shells of the snails were broken and the body was weighed. The whole of the reproductive system was dissected out as quickly as possible and weighed.

Light microscopy

Due to its large size the ovotestis, it was initially placed in the aqueous Bouin's fluid fixative (Drury & Wallingford, 1967) for 5 to 10 minutes to harden before the surrounding digestive gland tissues were carefully trimmed away. When the ovotestis was finally released, it was cut into thin strips of 1 mm with a very sharp razor blade and immersed in fresh fixative overnight. Sometimes the ovotestis and the tissues of the digestive gland were so joined to each other that freeing the ovotestis from the latter by careful trimming was very difficult and when this was the case, small pieces of the digestive gland and ovotestis were fixed together. Following fixation, the tissues were dehydrated in graded series of ethanol (50 – 100%) and cleared in xylene before embedding in paraffin wax. Serial sections of 4 to 6 μm thickness were cut on a rotary microtome. The sections were stained with haematoxylin and eosin.

Transmission electron microscopySmall pieces of tissues from the ovotestis were fixed overnight at 4 °C in 2.5% glutaraldehyde in 0.2M sodium cacodylate buffer containing 5% sucrose (pH 7.8) (Glauert, 1982; Reid 1982). Fixed tissues were rinsed in several changes of 0.2M sodium cacodylate buffer for 2 hours and post-fixed in 1% osmium tetroxide in sodium cacodylate buffer for 2 hours at room temperature. After rinsing the tissue in two changes of the same buffer, they were dehydrated in graded ethanol and embedded in Araldite via propylene oxide. Ultra-thin sections were cut using glass knives on a Riechert OM U3

ultramicrotome and picked up on copper grids. The sections were stained with uranyl acetate and Reynold's lead citrate (Reynolds 1963). The sections were examined on a Philips 400T transmission electron microscope.

Results

General morphology

The ovotestis of achatinids, including the species studied here, is an acinus organ containing male and female gametes in all stages of gametogenesis. The acini are numerous, closely grouped and were intimately associated with the digestive tissues. The wall of each acinus consists of pigment cells, a small amount of smooth muscle and fibrous connective tissues. In *Archachatina marginata ovum*, most acini contain only spermatogenic cells and only in a few are oocytes and spermatozoa found developing and maturing together (Figs. 1 and 2). Most stages of oogenesis were present in the acini within the gonad all year; however, oogenesis was asynchronous between the acini. Early oocytes are arranged close to the wall of the acinus where they develop until they fill its lumen.

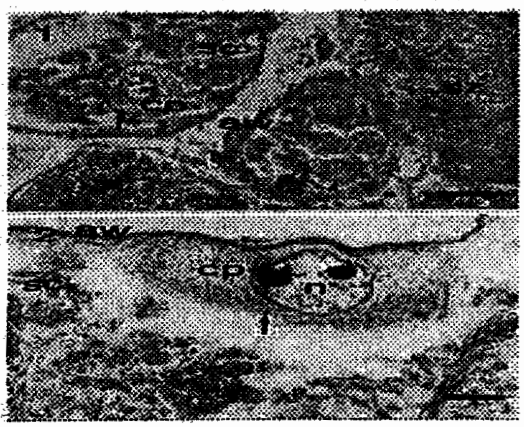

Figure 1: Light micrograph showing 3 acini of the ovotestis of *A. marginata ovum*. Note group of previtellogenic (arrow head) on the acinal wall (aw) and mature oocyte (arrow) in the lumen of acinus, with prominent nucleus (n) and extensive cytoplasm (cp). Also seen are spermatocytes (sc) and spermatozoa (sz) Scale bar = 40 μm.

Figure 2: Light micrograph showing early vitellogenic oocyte (arrow) of *A. marginata ovum* in which the nucleus (n) contains two nucleoli. Note the large surface area of oocyte in contact with acinal wall (aw). Note the wide cytoplasm (cp). Scale bar = 10 μm.

Premeiotic phase

The premeiotic phase consists of protogonia and oogonia. The protogonia cells (Fig. 3) are located on the acinal wall; they are round or elongated (3.6 μm in diameter) and appear to be in close contact with each other. The nucleus is oblong shaped with two nucleoli; it contains small patches of chromatin and is surrounded by a thin layer of cytoplasm. There were few scattered mitochondria and strands of rough endoplasmic reticulum inside the cytoplasm. There were two types of oogonia cell, primary and secondary (Fig 3). The primary oogonia are irregular in shape, measures about 4.3 μm in length with a large nucleus and a thin layer of cytoplasm. The cytoplasm contains mainly ribosomes, few mitochondria and strands of endoplasmic reticulum. Secondary oogonia are also elongated (7.0 x 3.3 μm), has an ovoid nucleus, and nucleolus was not observed. There were numerous large mitochondria, Golgi bodies and some endoplasmic reticulum found in the cytoplasm.

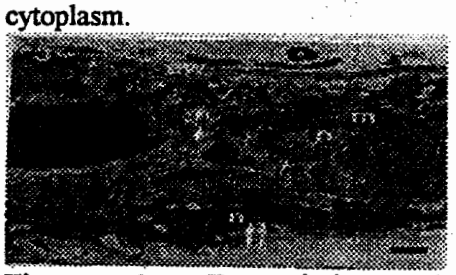

Figure 3: Transmission electron micrograph showing acinar cells of premeiotic protogonium (arrow head), primary oogonium (arrow) and secondary oogonium (two arrows) of *A. marginata ovum*. Protoogonia are attached to the inner

side of the acinar wall (aw). The oogonia are irregular in shape with oblong nucleus (n) and few small mitochondria (m). Scale bar = 20 µm.

Previtellogenic oocytes

The oocyte was enlarged compared to the oogonium in the premeiotic stage. Early previtellogenic oocytes (Fig. 4) are rounded and are located on the wall of the acinus, parallel to the basement membrane. It has a large rounded nucleus that has a nucleolus and measures 4.5 to 5.1 µm in length and 3.4 to 4.3 µm in diameter. The nucleus has a prominent nucleolus and scattered heterochromatin in the nucleoplasm. The cytoplasm of early previtellogenic oocyte is rich in free ribosomes and also contains some rough endoplasmic reticulum, small rounded mitochondria. Late previtellogenic oocytes are large and elongated. They have large nuclei containing randomly dispersed aggregates of heterochromatin scattered throughout the nucleoplasm and a prominent eccentric nucleolus. There are within the cytoplasm, Golgi bodies, mitochondria, few cisterna of rough endoplasmic reticulum, ribosomes, few lipid droplets, putative glycogen and few early yolk platelets. A single layer of developing follicle cells is seen around late previtellogenic oocyte; it completely surrounds the developing oocyte and separates it from the acinar wall (Fig. 5). Follicle cells are characterized by the presence of large quantity of rough endoplasmic reticulum, Golgi bodies, and there are many microvilli observed on the surface. As the oocytes grow, the nucleus develops more than one nucleolus. Unbranched microvilli, are observed on the surface of plasma membrane, some contact the follicle cells (fig. 5).

Vitellogenic oocytes

The oocytes continue to enlarge, but mainly in a plane perpendicular to the basement membrane (Fig. 6). Vitellogenic oocytes are elongated and completely enclosed by follicle cells which form a thin layer enveloping individual oocyte during development. Vitellogenic oocytes measure 12 to 14.2 µm in length and 8.1 to 9.3 µm in diameter in *A. marginata ovum* and are arranged on the wall of the acinus, protruding into the lumen. The nucleus of the oocyte increases in size to more than twice their original size; it is a large round nucleus with scattered heterochromatin and a single nucleolus. A considerable degree of cytoplasmic differentiation has also taken place. The cytoplasm of early previtellogenic oocyte contains numerous mitochondria, many cisterna of smooth endoplasmic reticulum packed with free ribosomes near the cell membrane. In the early stages, electron-dense granules are found near the Golgi body that may be the precursor of yolk. Yolk granules begin to appear once lipid formation starts; there are lipid droplets, often surrounded by putative glycogen granules in the periphery of the vitellogenic oocytes (Fig. 7). Many yolk platelets or granules are prominent in late vitellogenic oocytes (Fig. 8), and there are many cisterna of rough endoplasmic reticulum. As vitellogenesis proceeds, the vitelline coat becomes large and clear, the proteosynthetic organelles (rough endoplasmic reticulum and Golgi bodies) increase in number, mitochondria proliferate and elongate and are surrounded by complex arrays of rough endoplasmic reticulum. There is an increase in the number and size of the yolk granules which become uniformly electron dense, spherical, gradually fills the cytoplasm, eventually reaching a size of 0.2–0.7 µm in diameter.

Figure 4:Transmission electron micrograph showing early previtellogenic oocyte (arrow head) of *A. marginata ovum* on the acinal wall (aw). There is one nucleolus seen in the nucleus (n). There are seen in the cytoplasm small and round mitochondria (m), few lipid droplets (arrow). Scale bar = 1 μm

Figure 5:Transmission electron micrograph showing early vitellogenic oocyte. The eccentric nucleus (n) has one prominent nucleolus. There is a layer of follicle cells (f) around the oocyte (arrow). Note developing yolk platelets (arrow head). Note the microvillous brush border covering the oocyte surface (2 arrows) and projecting into the acinar lumen (al). There are few mitochondria (m), Golgi complex (g) and cisterna of rough endoplasmic reticulum (rer) and lipid droplet (arrow head) in the cytoplasm. Note the large surface area in contact with the acinal wall (aw). Scale bar = 1 μm.

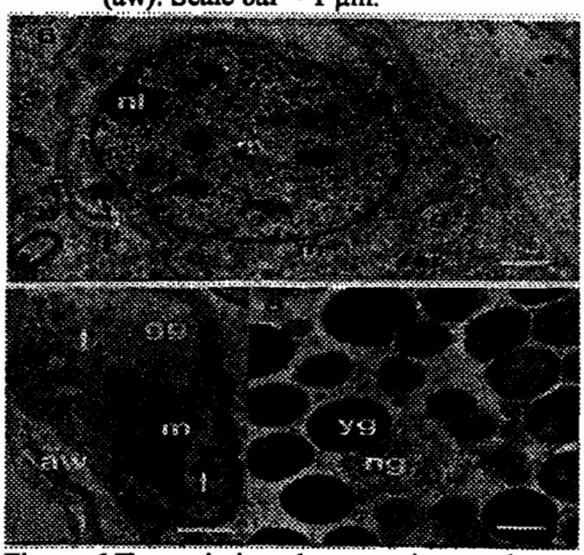

Figure 6:Transmission electron micrograph showing part of late vitellogenic oocyte in which yolk synthesis is under way and lipid droplets have begun to appear in the cytoplasm (arrow). The oocyte is on the acinal wall (aw). The nucleus (n) has one nucleolus (nl) and there is one prominent Golgi complex (g) in the cytoplasm. There is a layer of follicle cell (f) around the oocyte. Note the junction (2 arrow heads) between a follicle (f) and an oocyte (o). Scale bar = 0.5 μm. Abbreviations; ly, lysosome.

Figure 7: Transmission electron micrograph showng vitellogenic oocyte on the acinal wall (aw). Note extensive glycogen deposit (gg), mitochondria (m) and lipid droplet (l) in the cytoplasm. Scale bar = 1μm.

Figure 8: Transmission electron micrograph showing part of late vitellogenic oocyte of *A. marginata ovum.* Note stages of development of yolk. Scale bar = 2 μm. Abbrviations: yg, yolk granule; ng, nascent yolk granules.

Late vitellogenesis is the period prior to ovulation, when there are ripe eggs in the acinus lumen. Late vitellogenic oocytes measure 13.2 ± 1.2 μm in length and 8.7 ± 0.6 μm (n = 6). They are found on the acinar wall, there are many cisterna of rough endoplasmic reticulum packed with free ribosomes near the plasma membrane (Fig. 9). There are many yolk platelets or granules in the cytoplasm. The layer of follicle cells increase in size and contains numerous rough endoplasmic reticulum and some mitochondria. At maturity the oocyte detach from the germinal epithelium, the layer of follicle is removed and the unfertilized eggs then pass into the lumen of the acinus (Fig. 10).

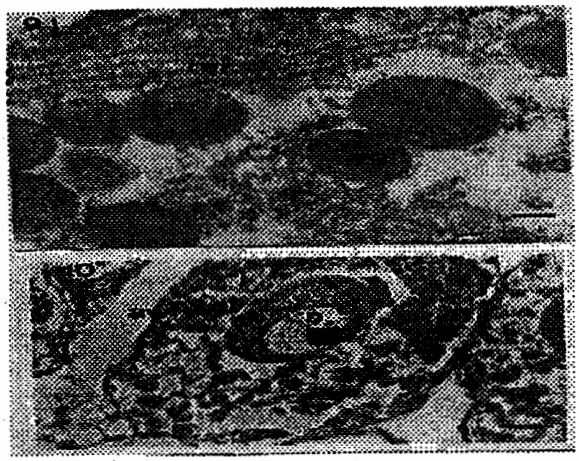

Figure 9:Transmission electron micrograph showing part of peripheral region of mature oocyte. Note the follicle cells pushed tightly against the plasmalemma of the oocyte (arrow). Note cisterna of rough endoplasmic reticulum (rer), formed yolk granules (yg) and lipid droplets (arrow). Scale bar = 0.25 µm.

Figure 10:Light micrograph showing mature oocyte (arrow) which has discharged from the acinal wall (aw) and lies free in the lumen. Scale bar = 40 µm.

Discussion

The sequential changes in ultrastructural morphology of *Archachatina marginata ovum* during oogenesis are reported in this study. The oocytes develop within the acini of the hermaphrodite ovotestis. The general features of the ovotestis of *A. marginata ovum* are similar to other pulmonates (Jong-Brink *et al.*, 1981; Luchtel *et al.*, 1997), male and female gametes develop within the numerous acini in the organ. In *Archachatina marginata ovum* the wall of an acinus was composed of connective tissues, muscles and epithelium which agrees with what has been described in other pulmonates (Sabelli and Sabelli-Scanabissi, 1982; Pal and Hodgson, 2002). Most acini contain only spermatogenic cells and in only a few are oocyte and spermatozoa found developing and maturing together. Oocyte development starts when the male gametes are at the spermatogonium-spermatocyte stage (Egonmwan, 2004). The production of oocytes was very quick and most of the snails examined hardly showed this process in contrast to spermatogenesis that lasted for a longer period. A complete female phase observed in *Arion ater* by Lusis (1961) was not observed in the species studied because at no time were the acini completely empty of sperm even if egg production was underway.

Three stages of oocyte maturation (previtellogenic, early vitellogenic and late vitellogenic) can be recognized in *A. marginata ovum*. Previtellogenic oocytes were initially few in number, later, both the number and size increased and they are most numerous at the spermatozoa–oocyte stage although only in few acini. They had clear nuclei with nucleoli and large cytoplasm, an indication that there is no mitotic activity at this stage, instead onset of cellular growth. Production of lipids and yolk occurs during the two vitellogenic stages in *A. marginata ovum*. Previtellogenesis and vitellogenesis are defined as the period from when the germinal cells become recognizable as oocytes, up to the stage when the first yolk precursor is formed (Kress, 1986).

Vitellogenesis is a complex process involving both autosynthetic and hetero-synthetic pathways that contribute to the formation of the yolk (Eckelbarger and Young, 1997). Results of the present study indicate that *A. marginata ovum* probably produce yolk granules by autosynthesis which was manifested by the increase in the number of rough endoplasmic reticulum, Golgi bodies and mitochondria. The involvement of rough endoplasmic reticulum and Golgi bodies and mitochondria in yolk formation has also

been reported in *Biomphalaria glabrata* (Jong-Brink *et al.*, 1976) and *Crassostrea virginica* (Eckelbarger and Young, 1997). Apart from yolk granules, lipids accumulate in the cytoplasm of *A. marginata ovum* during early vitellogenesis. Lipid was also been observed in the vitellogenic oocytes of *Siphonaria* (Pal and Hodgson, 2002), although the authors were also unable to determine how lipids were formed. There was very few yolk granules observed in the vitellogenic oocyte of *A. marginata ovum*. Gastropods lay eggs containing relatively small amount of yolk as the perivitelline fluid produced by the albumen gland provides nutrition for the embryo (Jong-Brink and Garaerts, 1982). In the freshwater snail *Biomphalaria glabrata* and *Lymnaea stagnalis* the yolk granules acquire lysosomal enzymes after oviposition and functions in the digestion of perivitelline fluid secreted by the albumen gland (Jong-Brink et al, 1976; Jong-Brink and Garaerts, 1982). The yolk granules of vitellogenic oocytes are differentiated into crystalline core surrounded by electron-lucent cortex in *Siphonaria capensis* and *Siphonaria serrata* (Pal and Hodgson, 2002), contain membranes in *Deroceras reticulatum* (Hill and Bowen, 1976) or contain both membranes and crystalloid inclusions in *Biomphalaria glabrata* (Jong-Brink *et al.*, 1976). In *A marginata ovum* no such differentiation was observed at this stage; the yolk was uniformly electron dense. There was a layer of follicle cells around the vitellogenic oocytes of *A. marginata ovum*, which became hypertrophic as development advanced due to the presence of rough endoplasmic reticulum, mitochondria and Golgi bodies in the cytoplasm. The closeness of the rough endoplasmic membrane of the follicle cells to the cell membrane of the developing oocytes suggests that the follicles cells probably play an important role in maturation of the oocyte in *A. marginata ovum*, although, the exact function was not determined during this study. Several functions have been suggested for follicle cells in molluscs (Taylor and Anderson, 1969; Bottke, 1974). In *Biomphalaria glabrata*, the follicle cells are involved in the formation of the follicular cavity during ovulation (Jong-Brink et al. 1976) and in the neritid gastropod, *Bathymerita naticoidea*, the follicle cells play a role in the nutrition of the oocytes and in the polychaete worm *Capitella*, the follicle cells were suggested as the possible site of extra-oocyte substances (Eckelbarger and Grassle, 1982). Joose and Reitz (1969) are of the opinion that Sertoli cells vacated by spermatozoa later become the follicle cells. The microvilli observed in the oocyte plasmalemma of *A. marginata ovum*, may be responsible for the absorption, transportation and secretion of egg envelopes (Pal and Hodgson, 2002), although it is not found in the oocytes of all molluscs (Eckelbarger and Young 1997).

During late previtellogenesis in *A. marginata ovum*, yolk formation is well established and mature oocytes are found in the period prior to ovulation, in the acinus lumen. The first phase of growth of oocyte is between previtellogenesis and early vitellogenesis and the final growth of the oocyte is between early and late vitellogenesis when the oocyte protrudes into the lumen of the acinus. Late vitellogenesis oocytes are found on the acinar wall where they are actively absorbing material from the haemocoelic blood vessel of the acinus (Hill and Bowen, 1976) and the structure in *A. marginata ovum* is similar to those of *Agriolimax reticulatus* (Hill and Bowen, 1976), *Mopalia mucosa* and *Chaetopleura apicula* (Anderson, 1969) and *Allotheutis subulata* (Bottke, 1974).

Ovulation takes place by withdrawal and partial autolysis of the follicle cells in *Lymnaea stagnalis* (Bretschneider, 1948) or by gradual withdrawal in *Siphonaria* (Pal and Hodgson, 2002), widening the gap between follicle cells and ultimately resulting in the bursting of the follicle and release of mature oocyte (Healy, 2001). The unfertilized eggs then pass into the lumen

of the acinus. This event was not observed in the present study, though some oocytes were seen in the lumen of acinus

The maturation of the gametes depended solely on the snails attaining a particular size and weight rather than on age. The oocytes of *A. marginata ovum* are very large contain large quantities of storage product to sustain the direct developing embryo.

Acknowledgements
I thank the two anonymous reviewers for their valuable comments on the manuscript.

References

Anderson, E. (1969). Follicle cell differentiation in two species of Amphineurans (Mollusca), *Mopalia mucosa* and *Chaetopleura apiculata*. *Journal of Morphology*. 125, 89–125.

Ajayi, S. S., Tewe, O. O., Moriarty, C. and Awesu, M. O. (1978). Observations on the biology and nutritive value of the giant African snail *Archachatina marginata*. *East African Wildlife Journal*. 16: 85-95.

Bequaert, J. C. (1950). Studies in the Achatininae, a group of African land snails. *Bulletin Museum of Comparative Zoology, Harvard*, 105, 1–216.

Bottke, W. (1974). Fine structure of the ovarian follicle of *Alloteuthis subulata* Lam. (Mollusca, Cephalopoda). *Cell and Tissue Research*, 150, 463–479.

Bretschneider, L. H. (1948). The mechanism of oviposition in *Limnaea stagnalis*. *Proceedings Koninklijke Nederlandse Akademie van Wetenschappen*, C51, 358-362.

Drury, R. A. B. and Wallington, E. A. (1967). *Carleton's Histological Technigue*, 4th Ed. Oxford University Press.

Eckelbarger, K. J. and Grassle, J. P. (1982). Ultrastructure of the ovary and oogenesis in the polychaete *Capitella jonesi* (Hartman, 1959). *Journal of Morphology*, 171: 305-320.

Eckelbarger, K.J. and Young, C.M. (1997). Ultrastructure of the ovary and oogenesis in the methane-seep mollusc *Bathynerita naticoidea* (Gastropoda: Neritidae) from the Louisiana slope. *Invertebrate Biology*, 116 (4): 299-312.

Egonmwan, R. I. (2004). Maturation timing in the land snails *Archachatina marginata ovum* (Pfeiffer) and *Limicolaria flammea* (Muller) (Pulmonata: Achatinidae). *Invertebrate Reproduction and Development*, 46: (2-3): 159-171.

Glauert, A. M. (1982). Fixation, Dehydration and Embedding of Biological Specimens. In: A. M. Glauert (ed.). *Practical Methods in Electron Microscopy*. Vol.3. Part 1, North-Holland Publishing Company, Amsterdam, Oxford. 207 pp.

Gomez, B.J. (2001). Structure and functioning of the reproductive system. In: G.M. Barker (ed). *The Biology of Terrestrial Molluscs*. CABI Publishing, Wallingford, New York. 307-355.

Healy, J.M. (2001). Spermatogenesis and oogenesis. In: G.M.Barker (ed). *The Biology of Terrestrial Molluscs*. CABI Publishing, Wallingford, New York. 357-382.

Hill, R. S. and Bowen, I.D. (1976). Studies on the Ovotestis of the slug *Agriolimax reticulatus* (Muller). 1. The Oocyte. *Cell and Tissue Research*, 173: 465-482.

Jong–Brink, M. de., Wit, A. de., Kraal, G. and Boer, H. H. (1976). A light and electron microscope study on Oogenesis in the freshwater pulmonate snail *Biomphalaria glabrata*. *Cell and Tissue Research*. 171: 195–219.

Jong-Brink, M. de., Boer, H. H. and Joose, J. (1981). Oogenesis, oviposition

and oosorption. In: K.G. Adiyodi and R.G. Adiyodi (eds.). *Mollusca: Reproductive Biology of Invertebrates*, Vol 1. John Wiley, New York, 297-355.

Jong-Brink. M. de. and Garaerts, W. P. M. (1982). Oogenesis in gastropods *Malacologia*, 22(1-2): 145-149.

Joose, J. and Reitz, D. (1969). Functional anatomical aspects of the ovotestis of *Lymnaea stagnalis*. *Malacologia*, 9: 101–110.

Kress, A. (1986). Ultrastructural study of oogenesis and yolk formation in an opisthobranch mollusc, *Runcina*. *Tissue and Cell*, 18(6): 915–935.

Luchtel, D. I., Martin, A. W., Deyrup-Olsen, I. and Boer, H. H. (1997). Gastropoda: Pulmonata. In: F. W. Harrison and A.J. Kohn (eds.). *Microscopic Anatomy of Invertebrates*. Wily-Liss, New York, 6B: 459-718.

Lusis, O. (1961). Postembryonic changes in the reproductive system of the slug *Arion ater rufus* L. *Proceedings of the Zoological Society, London*, 137: 433-468.

Mead, A. R. (1950). Comparative genital anatomy of some African Achatinidae (Pulmonata). *Bulletin of the Museum of Comparative Zoology, Harvard*, 105(2): 221-291.

Medina, A., Garcia, J. C. Moreno, F. G., and Lopez-Campos, J. L. (1986). Comparative studies on the histology of the ovotestis in the *Hypseldoris tricolor* and *Godiva banyulensis* (Gastropoda: Opisthobranchia), with special reference to yolk formation. *Journal of Morphology*, 188: 105-118.

Odiete, W. O. (1981). Histological observations on the ovotestis of the giant African land snail *Archachatina (Calachatina) marginata* Swainson (Gastropoda, Pulmonata, Stylommatophora). *Revue Zoologie africaine*, 95(3): 493–507.

Odiete, W. O. (1982). Fine structural studies on the ovotestis of *Archachatina marginata* Swainson (Pulmonata, Stylommatophora). *Malacologia*, 22(1-2): 137–143.

Pal, P. and Hodgson, A. N. (2002). An ultrastructural study of oogenesis in a planktonic and direct-developing species of *Siphonaria* (Gastropoda: Pulmonata). *Journal of Molluscan Studies*, 68: 337-344.

Pal, P. and Hodgson, A. N. (2005). Reproductive seasonality and simultaneous hermaphroditism in two species of *Siphonaria* (Gastropoda: Pulmonata) from the southeast coast of South Africa. *Journal of Molluscan Studies*, 71: 33-40.

Reid, N. (1982). Ultramicrotomy In: A. M. Glauert, (ed). *Practical Methods in Electron Microscopy*. Vol.3. Part 11, North-Holland Publishing Company, Amsterdam, Oxford. 353 pp.

Reynolds, E. S. (1963). The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *Journal of Cell Biology*, 17: 208–212.

Sabelli B. and Sabelli–Scanabissi, F. (1982). The structure of the acini of the gonad of *Ovatella myosotis* (Drapanaud) (Gastropoda: Pulmonata: Basommatophora)). *Monitore Zoologica Italiano*, 16: 333-339.

Taylor, G. T. and Anderson, E. (1969). Cytochemical and fine structural analysis ofoogenesis in the gastropod *Ilyanassa obsoleta*. *Journal of Morphology*, 129: 211-248.