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ORIGINAL PAPER

Note on the vitellogenesis and the yolk granules in a *Veronicellidae* slug, *Laevicaulis alte* (Férussac, 1822)

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Abstract

The *Laevicaulis alte* is a terrestrial, pulmonate slug. The ovotestis of the slugs comprises only one vitellogenic oocyte in each acinus. Ultrastructural features of the vitellogenesis and various yolk granules along with their different developing stages are described for the first time in the pulmonate slugs by a transmission electron microscope. Vitellogenesis accompanied by the autosynthetic and the heterosynthetic processes. Ooplasmic Golgi complex and rough endoplasmic reticulum are involved in autosynthesis, whereas endocytosis of microvillar process of the oolemma supports heterosynthesis of yolk bodies. The follicular cleft contains various secretory materials and glycogen granules, some of which are incorporated into the distal tips of the microvilli of the oolemma. In ooplasm, three types of yolk granules are discriminated morphologically. The Type-1 yolk granules are ovoid shaped, characterized by the presence of a well-developed axial band. The Type-2 yolk granules are oval-shaped filled with a uniform electron-dense matrix. The Type-3 yolk granules are circular and vacuolated, surrounded by few cisternae of the rough endoplasmic reticulum. Morphometric characteristics of the vitellogenic oocyte, follicle cells, and different yolk granules are noted. The number of yolk granules in the ooplasm of *L. alte* is compared to those of other pulmonate species in different habitats.

Keywords Follicular cleft · Follicular layer · Hermaphrodite · Ooplasm · Pulmonata · Types of yolk granules

Introduction

Yolk formation in the molluscs is a complex process including autosynthetic and heterosynthetic pathways with the formation of yolk granules in the oocytes (Favard and Carasso 1958; Recourt 1961; Jong-Brink et al. 1976; Griffond and Bolozoni-Sungur 1986; Kress 2003; Eckelbarger and Blades-Eckelbarger 1989). The ooplasmic Golgi complex and rough endoplasmic reticulum are usually involved in autosynthesis of yolk bodies while endocytosis of oolemma supports the incorporation of exogenous heterosynthetic yolk precursors (Barth and Jansen 1962; Jong-Brink et al. 1976; Griffond and Bolozoni-Sungur 1986; Kress 2003; Pal and Hodgson 2002; Kim 2016). The development of yolk granules accomplished by simultaneous coalescence of various yolk precursors such as yolk proteins (e.g. vitellogenin, ferritin, etc.), lipid droplets (e.g. phospholipids) and glycogen granules (Gérin 1976; Jong-Brink et al. 1983). The yolk materials of the pulmonates are almost similar, but there are some significant characteristics variations in their developmental patterns and the internal constituents of the yolk granules (Terakado 1974; Jong-Brink et al. 1976, Jong-Brink et al. 1983; Griffond and Bolozoni-Sungur 1986).

The ovotestis of the pulmonates contains numerous small ovoid-shaped acini. The male and female gametes are simultaneously developed in each acinus (Luchtel 1972; Bing et al. 2008; Roy et al. 2016, 2018). The ultrastructural characteristics of ovotestis have been studied in many hermaphrodite pulmonate molluscs in different habitats (Quattrini and Lanza 1964, 1965; Hill and Bowen 1976; Jong-Brink et al. 1976; Odiete 1982; Griffond and Bolozoni-Sungur 1986; Rakshit et al. 2005; Bing et al. 2008; Roy et al. 2016, 2018). However, the ultrastructural aspects of the vitellogenesis have been described in some aquatic pulmonates, but in terrestrial slugs are not so far described (Favard and Carasso

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1958; Recourt 1961; Terakado 1974; Jong-Brink et al. 1976; Khan and Saleuddin 1983).

The present study aims to describe detail ultrastructural characteristics of the vitellogenesis and the yolk granules in the oocyte of the terrestrial, pulmonate slug, *Laevicaulis alte* (Férussac, 1822). Morphometry of various yolk granules and various cell organelles in the ooplasm are documented. The numbers of yolk granules and their internal constituents in the ooplasm of *L. alte* are compared with the available data on the yolk granules of other pulmonate molluscs in different habitats.

The present study hypothesizes that the types and developing patterns of yolk granules are dependent on the species and the habitats of the pulmonates and may contribute to increasing a comprehensive understanding of reproductive strategies and taxonomy of the species.

Materials and methods

Species and study sites

The *Laevicaulis alte* (*Systellommatophora, Veronicellidae*, Pulmonata) was a terrestrial slug. Fifty slugs with a body weight of 1.02–1.08 g were collected from fields around the Kolkata (22.5726° N, 88.3639° E), West Bengal, India during the rainy season (June–July). The specimens were acclimatized in earthen pots and provided with leafy vegetables and water was sprayed regularly to maintain proper humid ambience.

Light microscopy

For light microscopy study, the ovotestis was dissected out from 25 living slugs and some samples of the ovotestes were fixed immediately in aqueous Bouin's solution in individual vials for 12 h, subsequently dehydrated and embedded in paraffin for 5 µm thick sections (Roy et al. 2016). These sections were examined under a light microscope (NIKON 50i) after staining with haematoxylin and eosin.

Transmission electron microscopy

For ultrastructural studies, some samples of the ovotestis from fifteen slugs were fixed immediately in a mixture of 3% glutaraldehyde and 2% paraformaldehyde in 0.1 M phosphate buffer (pH 7.2–7.4) in separate vials for 4–6 h or more at 4–6 °C. The ovotestes were then washed in several changes of 0.1 M phosphate buffer, post-fixed with 1% osmium tetroxide in double-distilled water for 2–4 h at the same temperature, dehydrated in an acetone series and embedded in an Araldite (CY 212) mixture (Roy et al. 2016). Semi-thin (1 µm) and ultra-thin (60–70 nm) sections were made with a Leica UC-7 ultramicrotome. The semi-thin sections were examined using a light microscope (NIKON 50i) after staining in 0.25% toluidine blue (Dykstra 1993). The ultra-thin sections were examined and photographed with Tecnai G^2 at 200 kV.

All electron-microscopical procedures were performed with the technical equipment of the 'Sophisticated analytical instrumentation facility', All India Institute of Medical Sciences (AIIMS), New Delhi, India.

The measurements of different cell organelles and the yolk granules (mean ± SD, $N = 3$) were made from transmission electron micrographs using 'ImageJ 1.51t' Software (Wayne Rasband, NIH, USA). The morphometry of the oocytes and follicle cells was done using light microscopy images.

Results

General description of the oocyte in the acini of *L. alte*

The acinus in the ovotestis of *L. alte* comprised of only one vitellogenic oocyte with some developing spermatogenic cells (Figs. 1, 2). The oocyte (4.91 × 3.39 µm in size) was surrounded by a layer (0.72 ± 0.12 µm in width) of follicle cells with a prominent follicular cleft or intermembranous gap between oolemma and follicular layer (Figs. 1, 2). The follicle cell (0.48 × 0.2 µm) was an oval-shaped, tiny epithelial cell with a prominent nucleus (Fig. 2). The follicular cleft was 0.46 ± 0.26 µm in width. The oocyte was composed

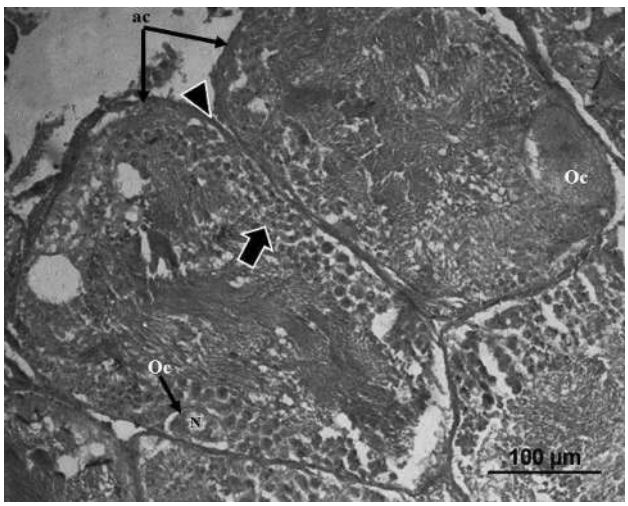

Fig. 1 Light microscope photograph (5 µ paraffin section) of ovotestis of *Laevicaulis alte*. Each acinus (ac) consists of one vitellogenic oocyte (Oc) with some spermatogenic cells. Arrow head- junction of acinar boundaries between acini. Bold arrow indicates spermatogenic cells. N, nucleus

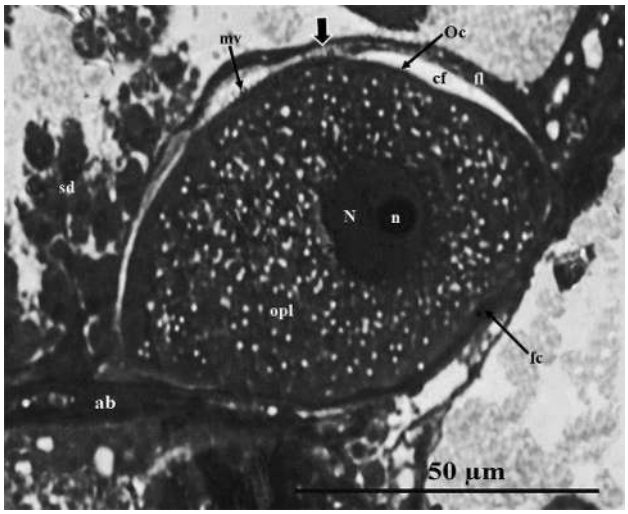

Fig. 2 Photomicrograph of semi-thin (1 µ) sections of a part of acinus showing magnified view of the oocyte (Oc). The follicular layer (fl) surrounds around the oocyte with a distinct follicular cleft (cf) between oolemma and follicular layer. Bold arrow indicates close association of follicular layer and microvilli of oolemma. *ab* acinar boundary, *fc* follicle cell, *mv* micro-villi, *n* nucleolus, *N* nucleus, *sd* spermatids

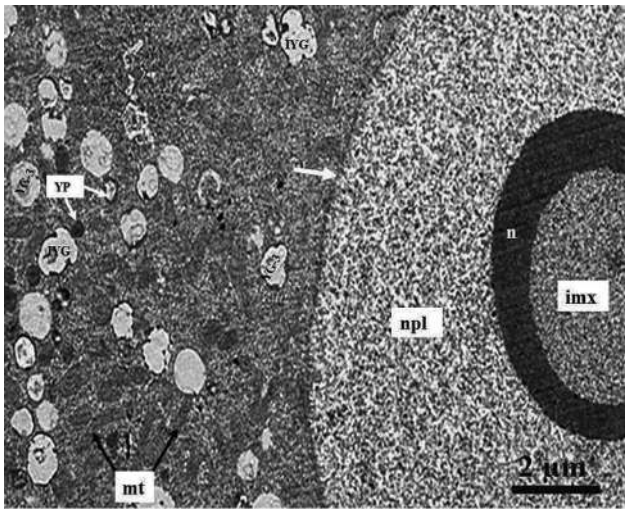

Fig. 3 Transmission electron micrograph of a part of oocyte showing ooplasm and nucleoplasm is separated by well-developed nuclear membrane with prominent nucleopores (white arrow). Nucleolus (n) consists of intranucleolar matrix (imx). *G* golgi complex, *IGY* immature or intermediate yolk granule, *L* lipid droplets, *mt* mitochondria, *npl* nucleoplasm, *YG-3* Type 3 yolk granule, *YP* yolk precursor

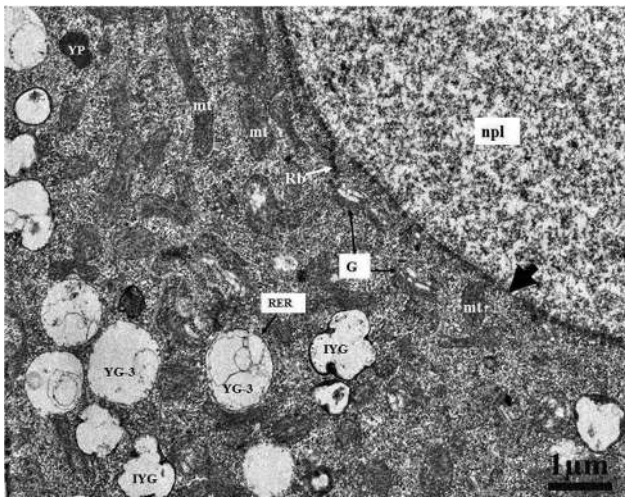

Fig. 4 TEM of a part of oocyte of *Laevicaulis alte*. Enlarged view of a part of ooplasm and nucleoplasm (npl), showing organization of ribosomes (Rb) on nuclear membrane and the cisternae of rough endoplasmic reticulum (RER) around the Type-3 yolk granule (Yg-3). Note the distinct different forms of mitochondria (mt) and nuclear pore (bold arrow head). *G* Golgi complex

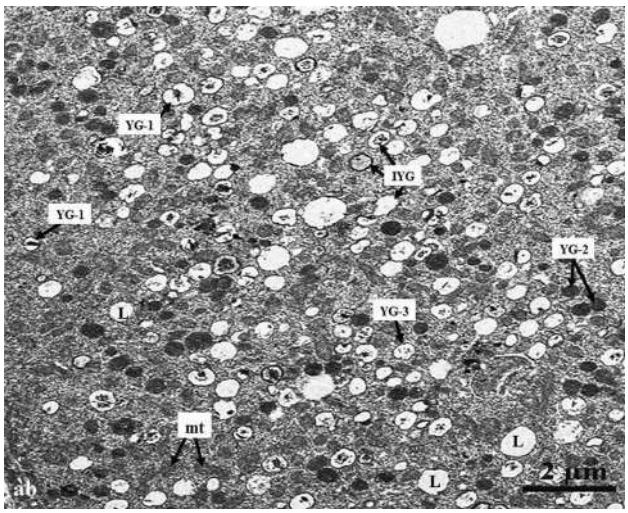

Fig. 5 Transmission electron micrographs of a part of ooplasm near acinar boundary showing various components in ooplasm. Note the populations of Type-1 (YG-1) yolk granules are comparatively lower than Type-2 (YG-2) and Type-3 (Yg-3) yolk granules. *ab* acinar boundary, *IYG* immature or intermediate yolk granules, *L* lipid droplets, *mt* mitochondria

of a large nucleus (1.97 ± 1.2 µm in diameter) with a prominent nucleolus with 0.60 ± 0.49 µm in diameter (Figs. 1, 2). The nuclear membrane of oocytes was smooth and exhibits high numbers of nuclear pores (Figs. 3, 4). Numerous electron lucent and electron-dense bodies were organized as a mosaic manner in the ooplasm (Figs. 2, 5, 6). Some parts of oolemma possess microvillar processes and few of which were closely associated with the follicular layer (Figs. 2, 7).

Vitellogenesis of *L. alte*

The vitellogenesis in *L. alte* was accomplished by both the autosynthetic and the heterosynthetic processes. The

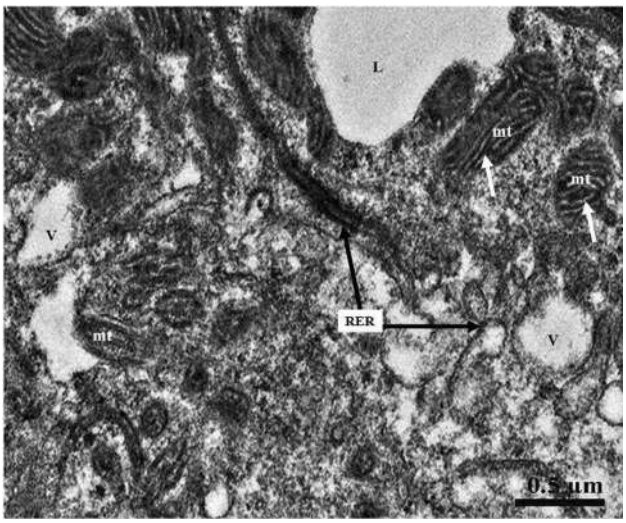

Fig. 6 TEM of a portion of ooplasm of *Laevicaulis alte*. Magnified view of rough endoplasmic reticulum (RER) and formation of vesicles (V) of different sizes. Note the well-developed cristae (white arrow) in mitochondria (mt)

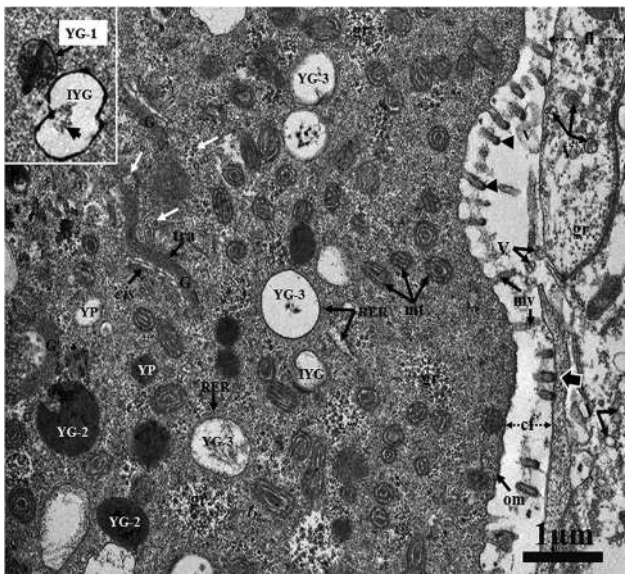

Fig. 7 Transmission electron micrographs of oocyte of *Laevicaulis alte*. An apical portion of the oocyte showing several yolk granules, various intermediate (or immature) yolk granules (IYG) and different yolk precursors (YP). The Golgi vesicles of various sizes are pinched off (white arrow) from the cisternae of Golgi complex (G). Note the prominent *cis*- (*cis*) and *trans*- (*tra)* face of the Golgi complex. Numerous mitochondria (mt) are distributed below the oolemma (Om) near the micro-villi (mv). The microvilli of oolemma leaned with small electron dense bodies (arrow head). Follicle cell layer (fl) comprised of glycogen granules (gr) and secretory vesicles (V) of various sizes. Typical deposition of yolk materials (arrow head) into yolk vesicle are shown in set. Bold arrow indicates the intermingled position of follicular layer and microvilli of oolemma. *cf* follicular cleft, *fl* follicular layer, *gr* glycogen rosette, *IYG* immature yolk granule, *om* oolemma, *RER* rough endoplasmic reticulum, *YG-1* Type-1 yolk granule, *YG-2* Type 2 yolk granule, *YG-3* Type 3 yolk granule, *YP* yolk precursor

ooplasm consists of several stacks of Golgi complex, mitochondria, rough endoplasmic reticulum, lipid droplets, and glycogen rosette. The stack of Golgi complex was small (0.18 ± 0.03 µm in width) and two to three are located together (Figs. 4, 7). The *cis* and *trans* face of Golgi complexes was prominent (Fig. 7). The *trans* face of Golgi cisterna discharges numerous, small moderately electron-dense vesicles (0.04 ± 0.02 µm in diameter) containing fine flocculent materials (Fig. 7). Some small vesicles (0.13 ± 0.02 µm in diameter) were found very close to the forming face of the Golgi complex involved in the formation of new Golgi cisterna (Fig. 7). Many small vesicles were simultaneously coalescence to form large vesicles (Figs. 3, 4, 5, 6, 7). The rough endoplasmic reticulum was either elongated (0.07 ± 0.03 µm in width of each cisterna) or vesicular in a form (Figs. 4, 6, 7). The numerous circular (0.23 ± 0.04 µm diameter) and elongated mitochondria (0.38×0.16 µm in size) were randomly distributed throughout the ooplasm (Figs. 6, 7). The mitochondria possess well-developed, swollen cristae (Figs. 3, 4, 5, 6, 7). A close topographical relationship between the Golgi apparatus, rough endoplasmic reticulum, and mitochondria was found in ooplasm (Figs. 6, 7, 8, 9a, b). The ooplasmic Golgi complex and rough endoplasmic reticulum were involved in the formation of yolk bodies (Figs. 6, 7, 8, 9a, b). Several ultrathin sections reveal that the yolk materials in ooplasm were represented by various forms of yolk precursors and different yolk granules (Figs. 3, 4, 5, 6, 7). Various yolk precursors were coalescence with

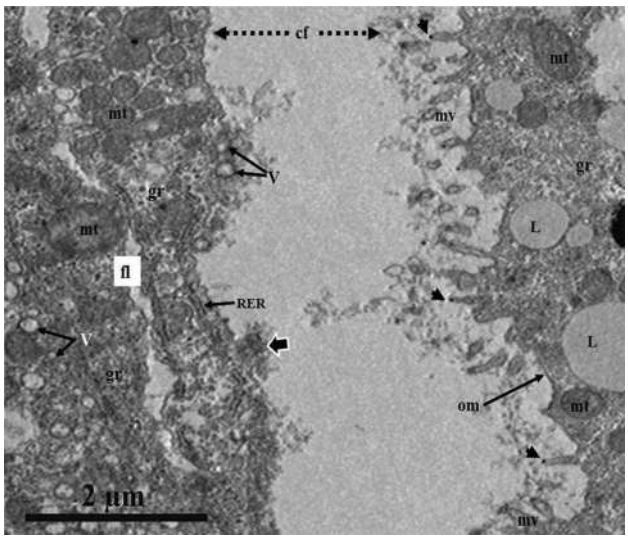

Fig. 8 TEM of a portion of oocyte of *Laevicaulis alte* viewing association of follicular layer (fl), follicular cleft (cf) and oolemma (om) of the vitellogenic oocyte. Small secretory vesicles (V) along with other cellular components (bold arrow) release into the follicular cleft. Note the incorporation (arrow head) of glycogen granules (gr) into the distal end of some microvilli (mv) are prominently observed. Numerous mitochondria (mt) and glycogen granules are found in both the follicular layer and oolemma near the area of endocytosis. *L* lipid droplets, *RER* rough endoplasmic reticulum

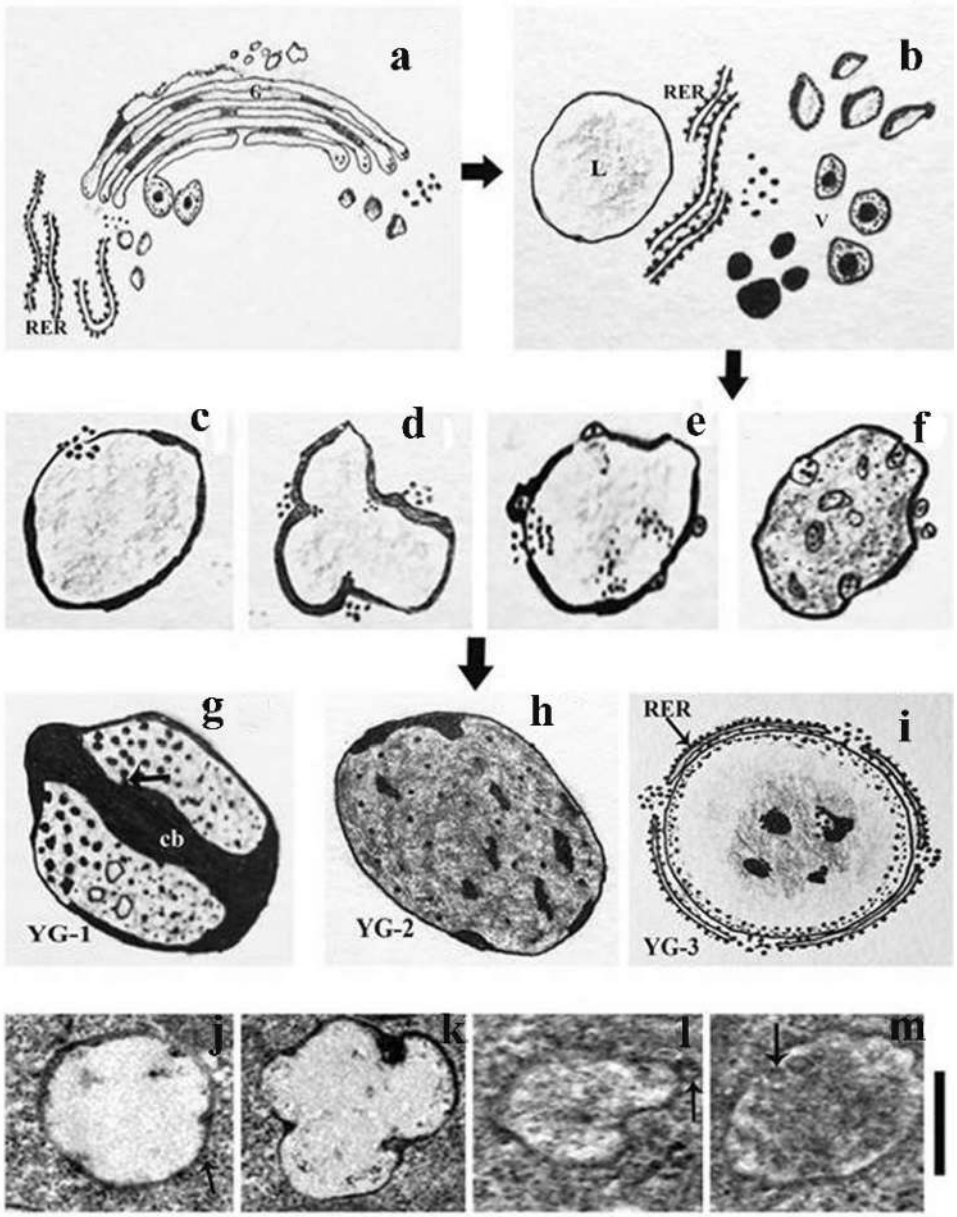

Fig. 9 A schematic outline of different yolk granules formation in the vitellogenic oocyte. **a** Involvement of Golgi complex (G) and rough endoplasmic reticulum (RER) in the formation of various yolk constituents; **b** various yolk precursors; **c–f** some forms of intermediate (or immature) yolk granules; **g** Type 1 yolk granule (YG-1). Arrow indicates arrangement of small electron dense granules in developing crystal band (cb); **h** Type 2 yolk granule (YG-2); **i** Type 3 yolk granule (YG-3). Bold arrow indicates the directions of the mechanism of yolk granules formation; **j–m** electron micrographs of some intermediate or immature yolk granules. Incorporation (arrow) of electron dense yolk bodies into the immature yolk granules (**j**); **k** anumber of small electron lucent vesicles is fused to form large vesicle; **l** small secretory vesicles coalescence (arrow) with large vesicle; **m** a large vesicle is filled with numerous small secretory vesicles (arrow) and electron dense materials; bold arrow indicates the directions of the mechanism of yolk granules formation. *L* lipid droplets, *RER* rough endoplasmic reticulum, *V* vesicles. Scale 1 µm

each other and produce different membrane-bound nascent yolk vesicles or immature yolk granules (0.30 ± 0.05 µm in diameter) with no definite shape and size (Figs. 3, 7, 8, 9c–f, j–m). The membrane of the immature yolk granules was gradually thickened due to the repeated fusion of small electron-dense yolk bodies and finally produces various mature yolk granules (Figs. 3, 4, 7, 8, 9c−f, j–m).

The follicular layer comprised numerous mitochondria, glycogen granules, small secretory vesicles and rough endoplasmic reticulum (Figs. 7, 8). The vesicles of the follicular layer contain fine flocculent materials. Several electron micrographs revealed that the glycogen granules along with other cellular materials of the follicular layer release into the follicular cleft (Figs. 7, 8). The distal ends of the microvilli of oolemma were inundated with various secretory materials in the follicular cleft (Figs. 7, 8). The incorporation of small electron-dense bodies into the distal tip of several finger-like microvilli was frequently observed (Figs. 7, 8). The heterosynthetic process of the vitellogenesis includes endocytosis of the microvillar process of oolemma (Figs. 7, 8). The morphometric characteristics of the oocyte and the follicular layer are presented in Table 1.

Yolk granules in the ooplasm of *L. alte*

There were three types of yolk granules in the ooplasm of the vitellogenic oocyte (Figs. 3, 4, 7, 10; Table 1). The electron micrographs suggest that the various small vesicles and

Table 1 Morphometric characteristics of the oocyte and the follicular layer of *Laevicaulis alte*

Characteristics	Measurement (μm)
Oocyte*	4.91 × 3.39 in size
Nucleus	1.97 ± 1.2 in diameter
Nucleolus	0.60 ± 0.49 in diameter
Ooplasm	
RER- cisterna	0.07 ± 0.03 width
Golgi complex	0.18 ± 0.03 in width
Cisterna	0.04 ± 0.01in width
Golgi vesicle	0.04 ± 0.02 in diameter
Glycogen rosettes	0.47 ± 0.17 diameter
Mitochondria	0.23 ± 0.04 diameter
Lipid droplet	0.84 ± 0.07 in diameter
Electron-dense vesicle	0.18 ± 0.25 in diameter
Follicular layer*	0.72 ± 0.12 in width
Follicle cell	0.48 × 0.2 in size
Follicular cleft	0.46 ± 0.26 in width
Mitochondria	0.58 ± 0.19 in diameter
RER-cisterna	0.09 ± 0.02 in width
Secretory vesicle	0.14 ± 0.07 in diameter
Micro-villi of oolemma	0.25 × 0.05 in size
Immature yolk granule	0.30 ± 0.05 in diameter
Moderately electron vesicles	0.18 ± 0.06 in diameter
Highly electron dense vesicles	0.25 ± 0.05 in diameter
Yolk granules	
Yolk granule-1	0.32 ± 0.04 in diameter
Yolk granule-2	0.43 ± 0.05 in diameter
Yolk granule-3	0.52 ± 0.09 in diameter

Values are mean ± SD ($N = 3$) with transmission electron microscopy. The asterix (*) viewed with light microscope

RER rough endoplasmic reticulum

electron-dense materials were fused and formed different types of yolk granules (Figs. 3, 4, 5, 6, 7, 9c–f, j–m, 10). The Type-1 yolk granules are ovoid shaped with 0.32 ± 0.04 μm in diameter. Each granule comprised highly electron-dense axial bands of crystalline materials. The formation of the axial bands was initiated in the electron-lucent vesicles and simultaneously developed by the repeated accumulation of small electron-dense granular bodies on both sites of developing axial bands (Figs. 5, 7, 9g, 10a1–a_4). The Type-2 yolk granules were oval-shaped with 0.43 ± 0.05 μm in diameter. The granules filled with a moderately electron-dense homogeneous matrix. Few irregular, highly electron-dense crystals were dispersed in the matrix of Type-2 yolk granules (Figs. 5, 7, 9h, 10b1–b_4). The Type-3 yolk granules were circular to semi-circular in shape, more or less transparent and vacuolated structure with 0.52 ± 0.09 μm in diameter. Type-3 yolk granules were comparatively larger than former two yolk granules and possess some filamentous as well as various granular materials. Type-3 yolk granules were characteristically surrounded by some cisternae of the rough endoplasmic reticulum (Figs. 3, 4, 5, 6, 7, 9i, 10c_1–c_4). The electron micrographs revealed that in ooplasm the population of the Type-1 yolk granules is lower than that of Type-2 and type-3 yolk granules. The developmental patterns, as well as various developing stages of these three types of yolk granules in the ooplasm, are represented in Figs. 9 and 10. The morphometric features of various yolk granules are represented in Table 1. The number of yolk granules and their internal constituents of *L. alte* was compared to that of the other pulmonate molluscs in Fig. 11 and Table 2.

Discussion

The observations of the present study describe the ultrastructural characteristics of the vitellogenesis and formation of various yolk granules in the *Laevicaulis alte*. The ultrastructural features of various yolk granules are described for the first time in a terrestrial pulmonate slug. It suggests that like other molluscs the yolk formation in the oocytes of *L. alte* accompanied by the autosynthetic and the heterosynthetic processes (Hill and Bowen 1976; Jong-Brink et al. 1976; Bottke et al. 1988). Various cell organelles involve together in the formation of lipid droplets, secretory vesicles, glycogen granules and yolk granules in the ooplasm. The ooplasmic Golgi complex and rough endoplasmic reticulum are involved in endogenous autosynthetic yolk formation. The endocytosis with the microvillar process of the oolemma is involved in exogenous heterosynthetic yolk formation as found in other pulmonates (Hill and Bowen 1976; Jong-Brink et al. 1976; Khan and Saleuddin 1983; Bottke et al. 1988). Several electron micrographs revealed that some small electron-dense granules are found in the microvilli and may support the endocytosis and transfer of extracellular yolk bodies into the vitellogenic oocyte of the *L. alte*. The coalescence of small vesicles and incorporations of glycogen granules into the developing yolk granules of *L. alte* involves in development of different mature yolk granules as reported in other pulmonates (Favard and Carasso 1958; Terakado 1974; Jong-Brink et al. 1983; Griffond and Bolozoni-Sungur 1986).

The nuclear membrane of oocytes exhibits high numbers of nuclear pores that may assist in cellular transportation between the ooplasm and the nucleoplasm. In ooplasm, the topographical relationship of Golgi complex, rough endoplasmic reticulum and mitochondria may assist in the formation of yolk precursors and the yolk granules (Favard and Carasso 1958; Terakado 1974; Jong-Brink et al. 1976; Griffond and Bolozoni-Sungur 1986). Numerous mitochondria with swollen cristae in the cortical area of the oocytes of *L. alte* (as seen in other pulmonates, e.g. *B. glabrata*,

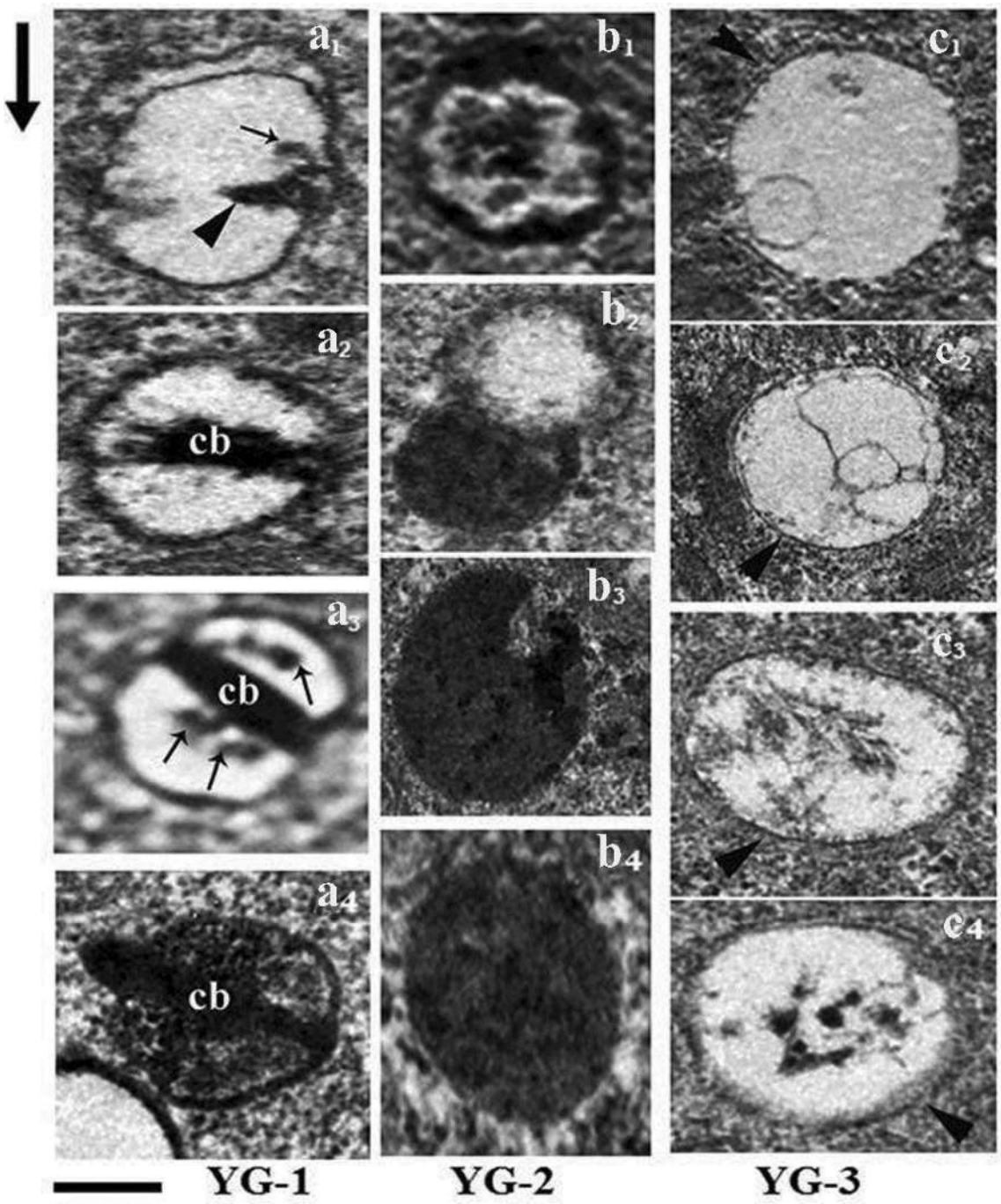

Fig. 10 A hypothetical arrangement of successive developing stages of three yolk granules (YG-1, YG-2, YG-3) in the ooplasm of *Laevicaulis alte*. Bold arrow indicates the directions of successive stages of three yolk granules; **a_1–a_4** representing the sequence of development of Type 1 yolk granule (YG-1). Arrow head indicates developing axial band (cb). Thin arrows indicate the incorporation of small electron dense granules in the developing axial band; **b_1–b4** representing the sequence of development of Type 2 yolk granule (YG-2); **c_1–c_4** representing the sequence of development of Type 3 yolk granule (YG-3); arrow head indicates rough endoplasmic reticulum (RER) around the Type 3 yolk granules. Scale 1 µm

Jong-Brink et al. 1976) may assist in endocytosis of exogenous yolk bodies. It is reported that in *Planorbarius corneus* (Favard and Carasso 1958) the yolk granules are formed from the rough endoplasmic reticulum, surrounded by cytoplasm but such kind of structures are not found in the oocytes of *L. alte* and other pulmonates (Jong-Brink et al. 1976, Jong-Brink et al. 1983).

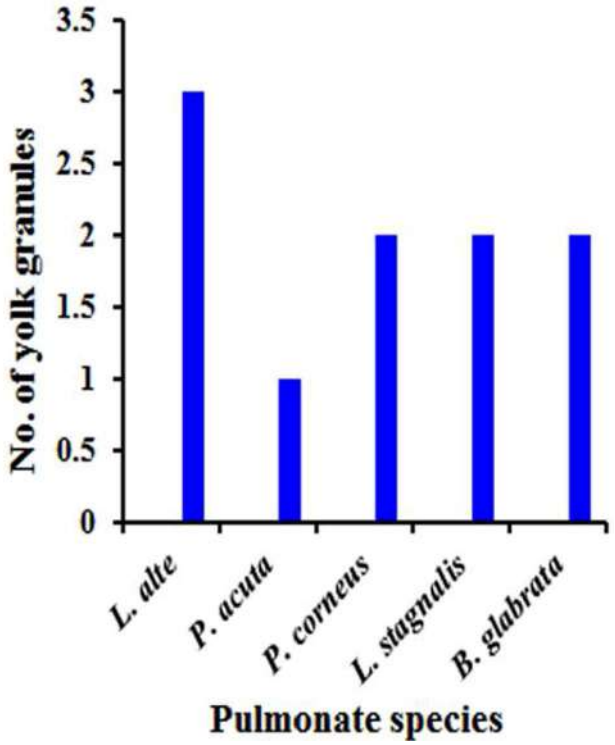

Fig. 11 The histogram showing the relative number of the yolk granules present in the ooplasm of terrestrial pulmonate *Laevicaulis alte* (*L. alte*) to aquatic pulmonates *Physa acuta* (*P. acuta*), *Planorbarius corneus* (*P. corneus*), *Lymnaea stagnalis* (*L. stagnalis*), and *Biomphalaria glabrata* (*B. glabrata*)

The follicle cell layer around the oocytes is a typical feature of gastropods (Jong-Brink et al. 1983; Eckelbarger and Young, 1997; Roy et al. 2016, 2018). The follicular layer consists of various yolk precursors, glycogen granules and secretory vesicles which may assist in the growth, nutrition as well as the vitellogenesis of oocytes (Recourt 1961; Taylor and Anderson 1969; Coggeshall 1970; Jong-Brink et al. 1976). The formation of the follicular cleft is an important feature of vitellogenic oocyte and is directly correlated to the development of the oocyte (Jong-Brink et al. 1976). The follicular cleft is filled with various cellular substances of follicle cells (Ubbels 1968; Jong-Brink et al. 1976).

The yolk granules of various pulmonate species exhibit certain morphological resemblances having crystalline content and vacuolated appearance, surrounded by rough endoplasmic reticulum (Elbers 1957; Jong-Brink et al. 1976, 1983). However, this is not established whether these morphologically similar yolk granules in different molluscan species are biochemically and functionally identical. It is also assumed that the developmental fashion of different yolk granules may be a species-specific event (Jong-Brink et al. 1976; Eckelbarger and Blades-Eckelbarger 1989; Pal and Hodgson 2002; Roy et al. 2018). In the oocytes of *L. alte*, three types of yolk granules are evident in the present study whereas, in *P. corneus* (Favard and Carasso 1958), *L. stagnalis* (Recourt 1961) and *B. glabrata* (Jong-Brink et al. 1976) possess only two types of yolk granules (Table 2). On the other hand, only one type of yolk granule is reported in the oocyte of *P. acuta* (Terakado 1974). It is advocated that the number of yolk granules in the ooplasm of the pulmonate molluscs is species-specific and may be influenced by their corresponding habitats.

Table 2 Relative features of the yolk granules and their major internal constituents viewed with transmission electron microscopy in various pulmonate molluscs in different habitats

Species name	Clades	Habitats	No. of yolk granules	Internal structures in yolk granules	References
Physa acuta	Hygrophila	Fresh water	1	Uniformly electron dense materials	Terakado (1974)
Planorbarius corneus	Hygrophila	Fresh water	2	Membranes and crystalloid inclusion	Favard and Carasso (1958)
Lymnaea stagnalis	Hygrophila	Fresh water	2	Electron dense bodies and crystalloid materials	Recourt (1961)
Biomphalaria glabrata	Hygrophila	Fresh water	2	Membranes and crystalloid inclusion	Jong-Brink et al. (1976)
Helix aspersum	Stylommatophora	Terrestrial	1/?	Uniformly electron dense materials	Griffond and Bolozoni-Sungur (1986)
Laevicaulis alte	Systellommatophora	Terrestrial	3	Moderately electron dense small vesicles, glycogen granules, electron dense filamentous materials and crystalloid materials	Present study

In *H. aspersum* the number of yolk granule is not clearly described (?)

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