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Ultrastructural Observations of the Vitelline Follicles and Vitellogenesis of *Acanthostomum* (*atrophocaecum*) *aswaninesis* Wannas, 1977 (Digenea, Acanthostomatidae), an Intestinal Parasite of *Bagrus bayad*

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Abstract: Vitellogenesis of *Acanthostomum (A.) aswaninesis, an intestinal parasite of Bagrus bayed, was studied for the first time in Egypt by transmission electron microscopy.* The ultrastructural observations indicated that, the vitellogenesis process is divided into four stages: Stage (I), immature vitellocytes have a small amount of cytoplasm, mainly filled with ribosomes and few mitochondria; stage (II), initiation of the synthetic activity, concerning an increase in the amount of endoplasmic reticulum, Golgi complexes and shell globule production; stage (III), active shell globule clusters synthesis; stage (IV), mature vitellocytes are filled with shell globule clusters, lipid droplets and α-glycogen rosettes particles. The general pattern and ultrastructure of vitellogenesis in *A. (A.) aswaninesis* greatly resembles those observed in other digeneans and some lower cestodes.

Key words: Platyhelminthes · Ultrastructure · Vitelline glands · *Acanthostomum (A.)aswaninesis*

INTRODUCTION

The platyhelminthes form a group of animals whose yolk supplies and shell proteins of the egg are not synthesized in the egg; it self, but also, in specialized cells called vitellines [1] and these cells are grouped in vitelline follicles.

Vitelline cells are believed to fill 2 main roles in egg formation, (1) synthesis of yolk for the developing embryo, (2) provision of a structural egg shell protein [2-4].

Vitellogenesis has been studied in various species of platyhelminthes, mainly in order Rhabodocela and subclass Eucestoda. In the Digenea, only 7 of the 24 superfamilies have been examined [5-7], including Diplostomoidea [8], Echinostomatoidea [2], Gorgoderoidea [9,10], Haploporoidea [11], Hemiuroidea [12], Microphalloidea [13], Schistosomatoidea [14], Cryptogonimiodea [15a,b] and Allocreadiidae [16]. According to the literature, many ultrastructural differences, as great morphological variability in the structure of vitelline cells between these families.

Yousfzai [17] stated that the egg-shell is formed by Mehlis gland, reinforced by vitelline granules which serve as nutriment.

The present study was conducted to reconstruct the ultrastructure of vitelline cells development in *A. (A.) aswaninesis,* an intestinal parasite of *Bagrus bayad*; and to compare the results with those reported for other species.

MATERIALS AND METHODS

Adult specimens of *A. (A.) aswaninesis* (Wannas, 1977) were collected a live from the intestine of naturally infected *Bagrus bayad*, caught from Timsah lakes.

Worms were removed from their hosts, fixed in cold (4°C) 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer at pH 7.2, rinsed in 0.1 M sodium cacodylate buffer at pH 7.2, post-fixed in cold (4°C) 1% osmium tetroxide in the same buffer for1 hour, dehydrated in ethanol and propylene oxide, embedded in resin at 60°C for 24 hours.

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Ultra-thin section (60-90 nm) of the worms, were cut using glass knives, in MT600 X L PMC ultramicrotome. Ultra-thin sections were mounted on copper girds and stained with uranyl acetate and lead citrate. Finally sections were examined on JEOL 1200 Ex2-trransmission electron microscope at the central laboratory, Faculty of Science, Ain Shams University.

RESULTS

The paired vitelline glands of *A. (A.) aswaninesis* cosnists of two lateral groups of a small oval vilelline follicles, distributed from the middle of the body and extending backwardly till the proximal end of the posterior testis, the vitelline follicles mainly overlap the intestinal caeca. Each follicle contains vitelline cells at various stages of maturation, that are situated close to each other, each follicle is enveloped by a small layer of intercellular tissue containing mitochondria and glycogen. Interstitial nucleated cells were observed surrounding the vitelline follicles (Figs. 1,3). Maturing cells in various stages of development found any where in the follicle, where mature cells tend to be located toward the center of the follicle (Figs. 1, 2).

Four stages of development have been observed from the immature to mature cells.

Stage (I) Immature Vitellocyte: Immature vitellocytes of *A. (A.) aswanensis* are irregular in shape with a prominent central nucleus, which occupied most volume of the cell. The nucleus often possesses a single nucleolus and patches of heterochromatin scattered through the nucleoplasm (Figs. 3,4). Early vitellocytes have a little cytoplasm, which is filled predominantly with free ribosomes, small number of mitochondria and some granular endoplasmic reticulum (Fig. 4). At this stage of development, the immature vitellocytes lack the characteristic shell globules and glycogen particles.

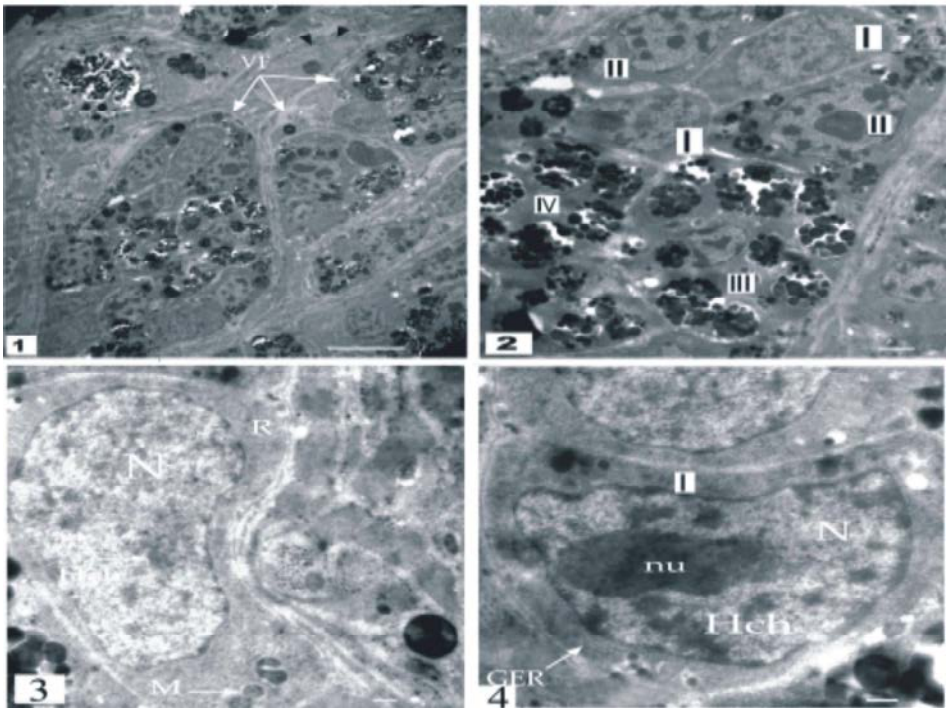

Fig. 1-4: Electron micrographs of a vitelline follicles and the first stage of vitellogenesis of *A.* (*A.*) *aswaninesis.*

(1) A vitelline follicles contains various stages of vitellogenesis, surrounding by interstitial tissue and nucleated cells (Arrow heads). Scale bar ~ 10 micron.

(2) Larger magnification of a vitelline follicle, contains cells at different developmental stages (I, II, III and IV) and their localization within the follicle. Scale bar ~ 2 micron.

(3,4) An immature vitellocyte (stage I) at the periphery of the follicle, containing a large nucleus (N) with a prominent nucleoleus (nu) and patches of heterochromatin (Hch). Cytoplasma of the cell filled with ribosomes (R); few endoplasmic reticulum (GFR); and mitochondria (M). Scale bar ~ 500 nm.

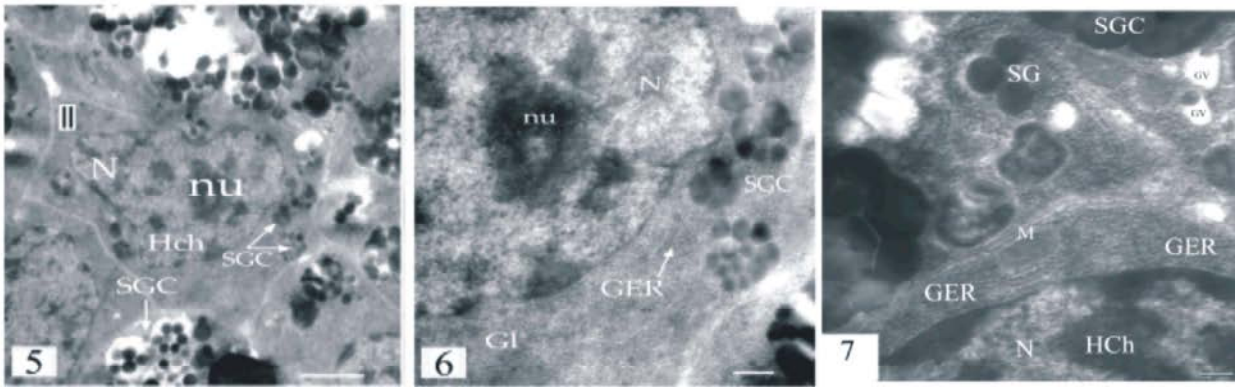

Fig. 5-7: Electron micrographs of a second stage of vitelline cells maturation.

(5) General observation of a vitelline cell at the second stage of maturation showing nucleus (N) with nucleolus (nu) and heterochromatin (HCh) and shell globule clusters (SGC). Scale bar ≃ 2 micron.

(6,7) Cytoplasm of a cell at the second stage of maturation filled with granular endoplasmic reticulum (GER) adjacent to nuclear membrane; a few enlarged, membrane-bound Golgi vesicles (Gv) containing moderately dense shell globules of different sizes; a few mitochondria (M); and glycogen (GL). Note the synthesis of a shell globule clusters (SGC) from single shell globule (SG). Scale bar ≃ 500 nm

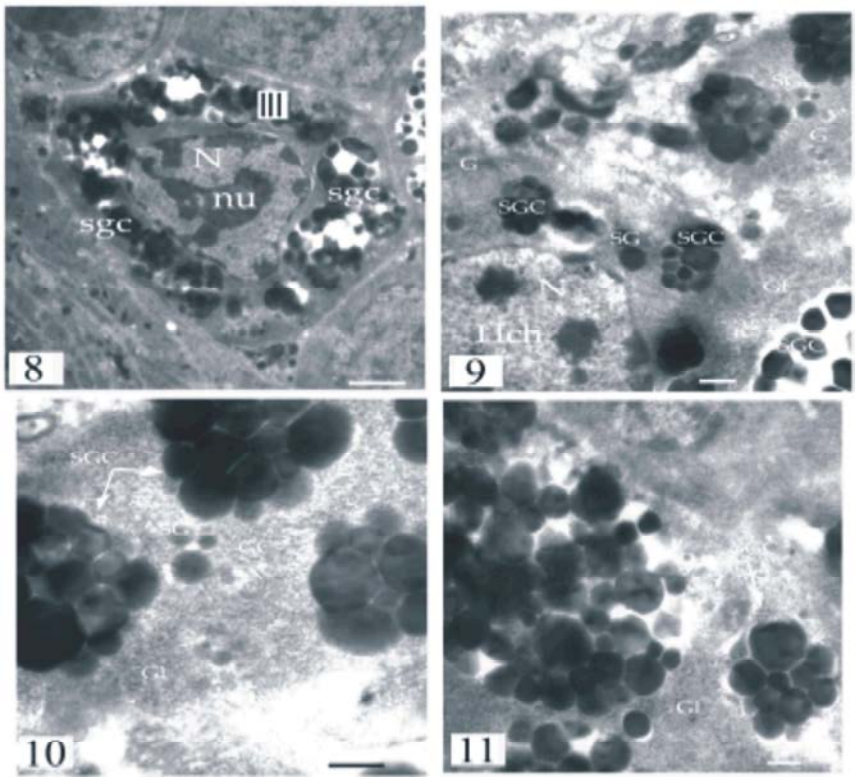

Fig. 8-11: Electron micrographs of the third stage of viteline cells maturation.

(8) General observation of the third stage of vitellien cell, showing nucleus (N) with nucleolus (nu) and shell globule cluster (SGc). Scale bar ≃ 2 micron.

(9,10,11) Cytoplasm of a cell at the third stage of maturation showing active synthesis of a shell globule (SG) and their fusion into large shell globule cluster (SGC); Golgi complex (GC) associated with shell globules; and glycogen particles (GL) around the shell globule clusters. Scale bar ≃ 500 nm

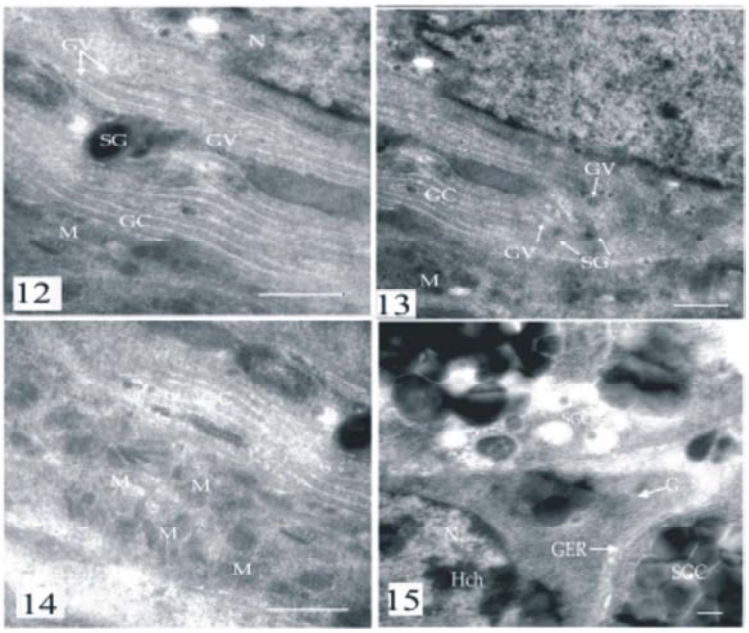

Fig. 12-15: Electron micrographs of the third stage of viteline cells maturation.

(12,13,14) Cytoplasm of a cell at the third stage showing enlarged Golgi cisternae (Gc) and vesicles (Gv). Note shell globule formation (SG) adjacent to Golgi cisternae and several mitochondria (M). Scale bar ≃ 500 nm

(15) Cytoplasm of a third stage vitelline cell showing great increase in development of (GER) and Golgi region (G) surrounding the shell globule clusters (SGC). Scale bar ≃ 500 nm

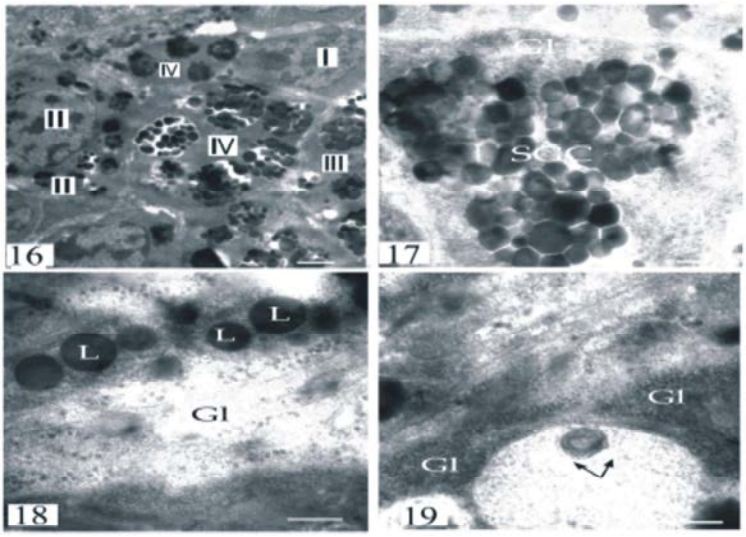

Fig. 16-19: Electron micrographs of the fourth stage of maturation of vitelline cells.

(16) General observation of vitelline cell at the fourth stage of maturation, containing a heavy accumulation of numerous shell globule clusters. Scale bar ≃ 2 micron.

(17) Details of peripheral cytoplasm showing numerous shell globule clusters (SGC), each containing a great number of individual densely packed shell globule and peripheral glycogen (GL). Scale bar ≃ 500 nm.

(18,19) A part of the cytoplasm with a lipid droplet and (L) and Rosette α-glycogen particles (GL). Note a concentric configuration of endoplasmic reticulum (Arrows), surrounded with glycogen (GL). Scale bar ≃ 500 nm.

Stage (II): Early Developing Vitellocytes: Early, when the cells begin maturation they increased is size, both the cytoplasm and nucleus increased in volume, but the nuclear-cytoplasmic ratio decreased. The nucleus contains electron-dense patches of heterochromatin and some of them settled on the nuclear membrane (Figs.5,6). The nucleoplasm becomes more denser than that of immature. The cytoplasm of early developing vitellocytes still contains mitochondria (Fig. 7); prominent granular endoplasmic reticulum, usually adjacent to the outer nuclear membrane and surrounding the shell globule clusters (Figs. 6,7); and shell globules, which appear to form singly, then aggregate, increasing in electron density and increasing in size to produce larger globules, that are grouped in shell globule clusters (Figs. 6, 7).

Glycogen has been observed at this stage of development located in the central cytoplasm (Figs. 6,7). Golgi vesicles were observed near the shell globule clusters (Fig. 7).

Stage (III): Advanced Developing Vitellocytes: During the vitelline cells development the nuclear-cytoplasmic ratio decreases. The chromatin is more condensed, larger and more settles on the nuclear membrane than the immature cells (Fig. 8). The characteristic feature of this stage is the presence of shell globules, that are grouped in clusters and surrounded by membranes (Figs. 8-11). In advanced developing vitelline cells, shell globule clusters continue their enlargement by fusion with single shell globules, that tend to be angular to optimize the space occupied (Figs. 8-11).

Golgi cisternae and vesicles occupies the entire cytoplasm in close associated with nuclear membrane and presence of several mitochondria (Figs. 12, 13, 14). Some Golgi cisternae are associated with small shell globules (Figs. 9, 10,15). Glycogen particles are observed around the vitelline clusters (Figs. 9,10, 11). At this stage, the endoplasmic reticulum are abundant and arranged in parallel around the shell globule clusters (Fig. 15)

Stage (IV): Mature Vitellocyte: Mature vitelline cells are generally localized in the center of the follicle (Fig. 16). The nuclei disappear, where they were not observed in mature vitellocyte. The cell cytoplasm is tightly packed with a heavy accumulation of numerous shell globule clusters, each containing a great number of individual densely packed shell globule and arranged at the periphery of the cell. In the most advanced stages, shell globules are separated by a fine electron-lucent matrix in the clusters (Figs. 16,17). The main feature of this mature stage is the appearance of a few electron-dense lipid droplets (Fig. 18). The glycogen particles are grouped in small amount around lipid droplets and shell globule clusters (Figs. 17,18,19). Some endoplasmic reticulum sacculis could be in a linear shape, or exhibit a concentric configuration (Fig. 19).

DISCUSSION

Vitelline cells at various stages of development were observed in each follicle of the vitelline gland. The vitellogenesis is basically the same in all Digenea [1,2,8-14,18-20], but some ultrastructural differences occur during the maturation, particularly with other platyhelminthes [4,21-27].

The maturation of vitelline cells of *A. (A.) aswaninesis* is divided into main four stages: stage 1, undifferentiated immature vitelline cells; stage 2, beginnings of synthetic activity; stage 3, active shell globule clusters synthesis; and stage 4, mature vitellocytes, like [3-16,20], who divided the process of vitellogenesis into 4 stages while, Sampour [11], Irwin and Threadgold [2], Grant *et al.* [8] and Irwin and Maguire [9] divided vittellogenesis into 3 stages. Fried and Hasseb [28] discussed that, a review of literature from 1975 to 1987 reveals that most authors follow the cleavage process for vitellogenesis of 4 stages for the platyhelminthes. Recently the latter method has been used for Trematoda [4,29]; Rhabditrophora [21,22,25]; Eucestoda [3,30]; and Monogenea [25,27]. Nevertheless, several special features can be distinguished between the different species of Digenea.

The nuclear cytoplasmic ratio decreases along the vitellogenesis due to the synthesis of large cytoplasmic inclusion, this is a closely similar to *Maritrema feliui* [20]; *Orientocreadium batrachoides* [16]; and *Metadena depressa* and *Aphallus tubarium*[15a,b]. The nucleus of vitelline cells of *A. (A.) aswaninesis* is ovale with irregular heterochromatin patches, generally distributed close to the nuclear membrane and the nucleolus has been observed from the first stage of maturation, this like to *Dicrocoelium dentriticum* [10] and *Maritrema feliui* [20], whereas in *Metadena depressa* [15a], only fully mature vitellocyte possess a large nucleolus and no nucleoli were observed in vitelline cells of *Aphallus tubarium* [15b] and *Pharyngostomoides procyonis* [8]. The fully mature vitellocyte of *A. (A.) aswaninesis* showing degenerating nucleus, this is closely resemble to *Maritrema linguilla* [13], *Maritrema feliui* [20] and *Orientocreadium. batrachoides* [16], but unlike to *Metadena depressa* [15a], *Aphallus tubarium* [15b] and *Haplopoprus lateralis* [11], in which the mature vitellocyte contain well developed nucleus with developed nucleolus.

The general structure of mitochondria of *A. (A.) aswaninesis* like that found in other digeneans [1,2,14-16,20]. Some differences manifest in the number of mitochondria during the maturation. Erasmus [14] observed the mitochondria are abundant in mature vitelline cells of *S. mansoni*. However in the present study, mitochondria were only, observed in the first three stages.

The presence of extensively developed granular endoplasmic reticulum and Golgi complexes in the second and third stages correspond to that observed in *Maritrema feliui* [20] and in *Orientocreadium batrachoides* [16].

Holy and Wittrock [12] and Erasmus *et al*. [19] discussed that, the attendance of endoplasmic reticulum, free ribosomes and Golgi complexes traduces a high synthetic activity that, give rise to shell globules, which coalesce to form clusters.

The mature vitelline cells of *A. (A.) aswaninesis* contains endoplasmic reticulum exhibit a spiral or concentric configuration, these structures are resemble those observed in *Aphallus tubarium* [15b]; *Orientocreadium batrachoides* [16] and *P. procyonis* [8] who called "membranous whorls" and discussed that, they may serve as a source of nutrition, or be residual bodies.

Shell globule clusters consider a characteristic feature in the process of vitellogenesis. Different forms of clusters have been observed during digenean vitelline cell studies. The shell globule clusters invitellocyte of *A. (A.) aswaninesis* are spherical in shape, they are very large in most mature stages (larger than the nucleus) and consists of tightly packed electron-dense globules combined with matrix of moderate electron density, unlike to those observed *Maritrema feliui* [20]; *Metadena depressa* [15a]; and *Orientocreadium batrachoides* [16], where the clusters consists of lossely packed electron-dense granules combined with electron-lucent matrix. However, the present clusters resemble those observed in *Aphallus tubarium* [15b] and in Caryophyllidean *Caryophyllaeus laticeps*; Spathebothriideans *C. trancatus*; and Pseudophyllidean *D. latumi* [31,32]. The shell globule cluster synthesis takes place in 3 stages for all platyhelminths. First the endoplasmic reticulum sacculis appear in the second stage of maturation. They are covered by ribosomes, reflecting a higher synthesis activity. Then the spherical Golgi cisternae give rise to shell globules [10,12,14].

The occurrence of lipid droplets and glycogen particles in the fully mature vitellocytes represent the nutritive reserves. For the future developing embryo [3,10].

In the present study, the production of lipid droplets occurs at the end of the maturation process, several electron-dense droplets were observed per cell, but this characteristic varies between platyhelminths, particularly for digeneanss, as in *Schistosoma mansoni*, several droplets were observed in the vitelline cells, whereas vitelline cells of *F. hepatica* [2] are devoid of lipid droplets. Both electron-dense and electron-lucent lipid droplets are present in the mature vitellocyte of *M. depressa* [15a]; electron-lucent lipid droplets were observed in mature cells of *Aphallus tubarium* [15b]; and electron-dense lipid droplets occurs in *Maritrema feliui* [20] and *Orientocreadium batrachoides* [16].

Levron *et al.* [4] reported that the production of glycogen is assumed to serve as source of energy for the developing embryo. In *A. (A.) aswaninesis* the glycogen synthesis begin in the second stage vitellocyte and present in the form of α-Rosettes particles in the fully mature vitellocyte, unlike to *M. linguilla* [13]; Schistosomes [19] *Maritrema feliui* [20]; *Orientocreadium batrachoides* [16] and *Aphaallus tubarium* [15b] in which glycogen occur only in single B-glycogen particles, whereas in *F. hepatica* the glycogen accumulated in both its forms [33,34]. In some digenean, there are no glycogen particles in vitellocytes during vitellogenesis.

The vitellogenesis of *A. (A.) aswaninesis* showing interstitial tissue, with mitochondria and glycogen particles surrounding the vitelline follicles, these structures are closely similar to those described in *Aphallus tubarioum* and *Metodena depressa* [15a,b] and *Halploporus lateralis* [11], but the present study revealed presence of nucleus in the interstitial cells, which has not been observed in the previous species. The nucleated interstitial cells of *A. (A.) aswaninesis* like to those observed previously in *Aspidogaster limacoides* (Aspidogastrea) where the nucleus occurs at the periphery of the vitelline follicles [4]; and also seen to correspond in structure to the nurse cells of *Fasciola hepatica* [2].

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