

Vitelline cells ultrastructure: experiment



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ULTRASTRUCTURE OF VITELLINE CELLS OF EXPERIMENTALLY RECOVERED MIGRATING *FASCIOLA GIGANTICA*

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ABSTRACT

The ultrastructure of migrating juvenile *Fasciola gigantica* shows that vitelline cells are grouped in vitelline follicles. Vitelline cells developed through a series of developmental stages. At stage I of vitelline cell development, the nucleo-cytoplasmic ratio is high and the cytoplasm has mitochondria. In the stage II, the cisternae of endoplasmic reticulum is appeared, the formation of shell protein globules is started and the cell increases in size, while the nucleo-cytoplasmic ratio decreases. At the final stage of development, stage III, the prominent structure is the domination of shell protein globules as the vitelline cell is fully matured and ready to deliver its shell protein globules to the fertilized eggs. This study is the first to show the fine structure of vitelline cells of migrating *F. gigantica* recovered from experimentally infected mice. The present work opens the way for more studies on experimentally recovered digenean worms and also exploring strategies for fighting the diseases caused by these worms.

Key words: Vitelline cells, migrating *Fasciola gigantica* and shell protein globules.

INTRODUCTION

Many ultrastructural studies were done on the different structures of *Fasciola* spp. like the tegument (ex. Threadgold, 1963 & 1967; Bennett and Threadgold, 1975; Skuce and Fairweather 1989. Hiekal, 1992; Stoitsova and

Gorchilova, 1997; Sobhon et al., 1998; McKinstry et al. 2007; Stoitsova and Gorchilova, 2010). The caecal epithelium (ex. Thorsell and Björkman, 1965; Gallagher and Threadgold, 1967; Halton, 1967; Bennett and Threadgold, 1973; Robinson and Threadgold, 1975; Bennett, 1975; Davies, 1978; Threadgold, 1978; Ashour et al., 2001a; Meemon et al. 2010 and Savage *et al.* 2014).

Spermatogenesis of adult *F. gigantic* was studied by Ashour et al., (2001b) and Ndiaye et al., (2004). Ashour et al., (2003) also studied spermatogenesis and spermiogenesis of migrating *F. gigantica*; in that study, Ashour and his colleagues (2003) demonstrated that the migrating *F. gigantica* worms recovered from body cavity of experimentally infected mice reach their maturity; as all spermatogenesis and spermiogenesis stages were observed in these migrating worms.

Some studies were done on the female reproductive system and different structures of adult female *Fasciola* spp. that recovered from naturally infected hosts Holy and Wittrock, (1986). Many studies on the structure and development of vitelline cells and egg shell formation were done by many authors like Stephenson (1947), Yosufzai (1953) Burton 1963, Bjorkman and Thorsell (1963), Irwin and Threadgold (1970 and 1972), Hendow and James (1989), Colhoun et al. (1998), Fawzy et al. (2000) Meepool et al. (2006) and Savage *et al.* (2014).

It was reported in some digenean trematodes, that the vitelline follicles contain vitelline cells in various stages of development (Stephenson, 1947; Skuce and Fairweather; 1988 add some refs Wells & Cordingley, 1991;

Colhoun et al., 1998; Robinson et al. 2001, and Meepool et al. 2006). The mature vitelline cells are derived from stem cells in the vitelline follicles (Stephenson, 1947, Irwin and Threadgold, 1972, Threadgold, 1982 and Colhoun et al., 1998) add some refs. It was shown that the synthesis of shell protein globules begins in the maturing vitelline cells of the digenetic trematode *Haploporus lateralis* (Sampour, 2008), *Opisthorchis viverrini* (Khampoosa et al, 2012). The egg shell precursor proteins are synthesized by the vitelline cells (Threadgold, 1982). The vitelline cells also provide the developing embryo with nutrients in the form of glycogen and yolk material (Threadgold, 1982; Martinez-Alos *et al.* 1993 and Swiderski and Xylander 2000).

In the present study, the ultrastructure of the vitelline cells of migrating juvenile *F. gigantica* worms are demonstrated for the first time.

MATERIALS AND METHODS

Worm collection : Adult worms of *Fasciola gigantica* Cobbold, 1855 were collected from the bile ducts of buffaloes (*Bubalus bubalis*), at Basateen abattoir, Cairo. These worms were transferred into a warm saline solution (0.85% NaCl) for oviposition. Deposited eggs were washed with distilled water and were incubated at 26 – 28°C.

Snail infection : After 14 – 21 days post incubation, eggs hatch into miracidia. Laboratory bred snails *Lymnaea natalensis* Krauss, 1848 were individually put into vials with 3 – 5 miracidia and left for 24 hours to guarantee the snail's infection. After 35 days post infection (pi), the cercariae were shed out from the infected snails, and then encysted on the vials' walls and

bottoms forming metacercariae. The metacercariae were genety collected, counted and maintained in clean vials with distilled water and stored at 4i,°C.

Mice infection : Mice were obtained from Schistosoma biological supply program (SBSP) unit at Theodor Bilharz institute, Giza. Mice were starved 24 hours before infection then fed individually on a small piece of bread containing metacercariae. After 50 days pi, mice were dissected and carefully examined for the presence of any *F. gigantica* worms in liver or even in the peritoneum.

Electron microscopy : Worms were sliced transversely into halves and fixed in 3% glutaraldehyde for two hours at room temperature, specimens were washed 2 - 3 times in phosphate buffer (pH 7. 2), and post fixed in 1% osmium tetroxide for two hours at 4i,°C then washed in the phosphate buffer. Specimens were dehydrated in an ascending series of ethanol then acetone and then embedded in resin (Epon 812). Ultrathin sections were stained with uranyl acetate and lead citrate and examined under JEOL (JSM-6300) transmission electron microscope at the regional centre of fungi, El Azhar University, Cairo.

RESULTS

Vitelline cells aggregated in vitelline follicles (Figures 1, 2 and 3), and contained the shell protein (vitelline) clusters which are peripherally arranged underneath the cell membrane of the mature vitelline cells (Figure 6). Shell protein clusters surrounded the nucleus of vitelline cell in a circular manner (Figure 7). The shell protein clusters were formed from many

accumulated smaller shell protein globules (Figures 5 - 10). The number and size of shell protein globules in the vitelline clusters varied among different clusters as they ranged from 1 to 15 globules (8.62 ± 3.67). Vitelline cells are active cells due to abundance of rough endoplasmic reticulum (RER) within their cytoplasm; RER is concentrated around the nucleus (Figures 8 and 9). The stem cell (Figure 7 & 13), the cell from which the vitelline cells were differentiated, measured $8.14 \times 3.14 \mu\text{m}$, with a very large nucleus (6×2.71) that occupies most of the cytoplasm and contains aggregated chromatin. Vitelline cells were differentiated into three developmental stages: I, II and III.

In stage I, the cell was elongated and reached $13 \times 3.4 \mu\text{m}$; the nucleus was elongated as well and reached $6.6 \times 2.6 \mu\text{m}$. The nucleus occupied most of the cell (high nucleo-cytoplasmic ratio) and contains high content of chromatin (Figure 6 & 14). Many elongated mitochondria were also observed in the cytoplasm (Figures 4 & 5); these mitochondria have obvious cristae (Figures 10 - 12).

In stage II of development (Figures 6, 7, 10 & 15), the cell reached to $13.73 \times 8.4 \mu\text{m}$. The nucleus ($6 \times 4.26 \mu\text{m}$) was gradually displaced from the cell's centre and the chromatin aggregations started to decrease. On the other hand, the formation of shell protein globules is started; globules are accumulated either at one pole of stage II cells or around their nuclei (Figures 6, 7 & 15). In this stage of development, RER is found and surrounding the nucleus; the endoplasmic reticulum are formed from many parallel cisternae which take a perinuclear position (Figures 7 & 8).

The stage III of development, vitelline cells (19 x 9 μm) represents the mature vitelline cells which are filled with shell protein globules in the form of clusters; each cluster was formed from globules of various sizes and arranged beneath the cell membrane and surrounds an electron-lucent matrix (figures 1, 3, 6 & 16). The nucleus started to decrease in size (Figure 10) and the electron-lucent matrix started to be formed. The individual shell protein globules attached to each other within the cluster and the number of shell protein clusters is varying among mature vitelline cells as they ranged from 7 to 18 (12.25 ± 3.89) cluster per cell. The cluster size varied in the mature vitelline cells as it ranged from 0.25 to 0.54 μm (0.34 ± 0.07). The lowest diameter of shell protein globules is 0.074 μm ; while the highest diameter of shell protein globules is 1.48 μm which means that the largest shell protein globules is 20 times the smallest shell protein globules. Mitochondria and endoplasmic reticulum are absent from the mature vitelline cells (stage III of development). Yolk granules were also observed in vacuoles within the electron-lucent matrix of the stage III of vitelline cells (Figure 3)

DISCUSSION

The high egg production in the liver fluke *F. hepatica* with its high number of vitelline cells revealed that, *F. hepatica* has to consume and produce materials for its reproductive needs (Meepool and Sobhon, 2009). In the present work, the finding of a large number of vitelline cells and a great number of clusters of shell protein globules may indicate high rates of egg production in *F. gigantica* as well; the presence of prominent RER in the cytoplasm of vitelline cells indicates a higher activity of protein production

and this is in agreement with the findings of Irwin and Threadgold (1972) and Colhoun et al. (1998). In the present study the large number of individual globules within the shell clusters also indicates a high rate of egg production that needs a continuous supply for the shell formation (protein material) which is translated in the abundance of RER in the vitelline cells.

In *F. hepatica*, it has been estimated that the adult worm produces ~ 25, 000 eggs / day (egg / 3. 46 sec.) Happich and Boray (1969); According to Meepool and Sobhon (2009), each egg consists of one ovum and about 30 vitelline cells and as it was recorded by Happich and Boray (1969) that an oocyte was produced every 3. 46 sec., so one vitelline cell will be produced every ~ 0. 12 sec.(Meepool and Sobhon, 2009). When the shell protein globules of *Fasciola hepatica* are formed they migrate to the periphery of vitelline cells where large clusters accumulate there and a certain degree of globules' fusion within these clusters is reported (Irwin and Threadgold, 1972). In the present study the coalescing of shell protein globules in clusters may happen in a similar process to that reported by Irwin and Threadgold (1972). The presence of shell protein globules at the cell periphery indicates that the vitelline cells are mature and they are ready to discharge the protein globules onto the newly formed eggs to form their shells.

Eggshell production in *Schistosoma mansoni* has been proposed by Wells & Cordingley (1991), as eggshell protein globules were released of from the vitelline cells within the ootype then followed by their subsequent fusion to form the eggshell; Fusion and tanning of these components produces eggshell. Egg formation in *F. hepatica* takes place in the ootype, which is <https://assignbuster.com/vitelline-cells-ultrastructure-experiment/>

surrounded by the Mehlis' gland cells. Each egg involves the combination of an ovum, approximately 30 vitelline cells and secretions from the Mehlis' gland. The vitelline cells release shell protein globules which coalesce around the cell cluster to form the eggshell Colhoun et al. (1998). The results of Colhoun et al. (1998) suggest that the mechanism for eggshell formation in *F. hepatica* is similar to that proposed for *S. mansoni* by Wells & Cordingley (1991) and may be common to other trematodes as well.

Greani *et al.* (2012) studied the ultrastructural organization of the female reproductive system of *Metadana depressa*, digenean intestinal parasite of Sparidae (*Dentex dentex*), and they found that, vitellogenesis is divided into four stages: stage I, vitellocytes have a cytoplasm mainly filled with ribosomes and few mitochondria; stage II, beginning of the synthetic activity; stage III, synthesis of active clusters of shell globules; stage IV, maturation of vitellocytes which are filled with shell globule clusters and generally contain several large lipid droplets. Glycogen granules are grouped at the periphery of the cell. In the present study, the development of vitelline cells occurs through three stages; stage I with large nucleus and some mitochondria, with no ribosome were observed. In stage II, the synthetic activity of shell protein globules are started and appearance of endoplasmic reticulum loaded with ribosomes. In stage III, shell protein clusters are the dominated structure with electron-lucent matrix; so the developmental stages of migrating *F. gigantica* and *Metadana depressa* are nearly similar as both worms are digeneans and this may confirm the hypothesis of Colhoun et al. (1998) for the similarity of vitelline cell development in trematodes.

In the present study, the average number of the shell protein globules within the cluster was about 8 globules which much lower than those counted by Greani *et al.* (2012) in their study of vitelline cells of *Met adena depressa* (intestinal digenean of *Dentex dentex*) as they recorded 45 globules per cluster. On the other hand the average cluster diameter of vitelline cells of the present study is smaller (0. 34 μm) than that of vitelline cells of *Metadena depressa* (3 μm) (Greani *et al.* 2012). The shell protein globules in the present study are attached together in the form of spherical cluster; while shell protein globules are individually grouped in clusters in vitelline cells of *Metadena depressa* (Greani *et al.* 2012).

In the present study, the nucleo-cytoplasmic ratio is decreased with vitelline cell development; this observation was noticed in the development of vitelline cells of *F. hepatica* Colhoun *et al.* (1998) and *Metadena depressa* Greani *et al.* (2012). In migrating *Fasciola gigantica* , the electron-lucent matrix is greater than that in *M. depressa* .

CONCLUSION

The present study proved that the migrating *F. gigantica* worms that recovered from experimentally infected mice can reach maturity, produce eggs and have normal development of vitelline cells. This work also represents a new trend for more studies on the reproductive organs of digenetic trematodes from experimentally infected animal models.

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