

Histology and Ultrastructure of the Vitelline Gland of the Pancreatic Fluke *Eurytrema pancreaticum*

Jantane Boonameepool¹ and Jirat Boonameepool^{2*}

¹ Faculty of Science, Burapha University, Thailand

² Faculty of Allied Health Sciences, Burapha University, Thailand

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Abstract

Background and Objectives: The vitelline gland is an essential reproductive structure in trematodes, with vitelline cells contributing to eggshell formation and providing nutrients and other components required for embryonic development. During vitellogenesis, developing vitelline cells undergo a series of morphological and ultrastructural changes associated with the synthesis and accumulation of specific cytoplasmic inclusions, particularly eggshell globules and lipid droplets. The composition and organization of these inclusions vary among digenean species and may reflect differences in reproductive strategies and patterns of egg development. In trematodes that produce embryonated eggs, vitelline cells may exhibit specialized structural characteristics associated with embryonic development prior to egg release from the definitive host. However, detailed information on the ultrastructural differentiation of vitelline cells in trematodes that produce embryonated eggs remains limited. In particular, the structural relationship between developing vitelline cells and their associated nurse cells has not been extensively characterized. To address this gap, this study investigated the histological organization and ultrastructure of the vitelline gland of *Eurytrema pancreaticum*, an embryonated egg-producing digenean trematode, with particular emphasis on the sequential differentiation of vitelline cells and the structural characteristics of nurse cells during vitellogenesis.

Methodology: Vitelline glands from five adult *E. pancreaticum* specimens were processed for resin embedding and examined using light microscopy (LM) and transmission electron microscopy (TEM). Semithin sections were examined by LM to characterize the organization and distribution of vitelline follicles. Ultrathin sections were examined by TEM to characterize the ultrastructural features of vitelline cells at different developmental stages and their associated nurse cells. Vitelline cells were classified into developmental stages based on their cytoplasmic organization and the presence, size, and distribution of characteristic cytoplasmic inclusions. Ultrastructural assessment focused on eggshell globule clusters, lipid droplets, glycogen particles, glycan vesicles, cellular junctions, and the association between vitelline cells and nurse cells.

Main Results: The vitelline gland consisted of numerous follicles arranged in two lateral groups on either side of the parasite. Each follicle contained developing vitelline cells surrounded by flattened nurse cells. Based on

their ultrastructural characteristics, vitelline cells were classified into four developmental stages: immature, intermediate type I, intermediate type II, and mature cells. Immature vitelline cells exhibited relatively undifferentiated cytoplasm with early development of characteristic inclusions. During intermediate differentiation, eggshell globules progressively appeared, increased in number and size, and became organized into distinct clusters. Lipid droplets also accumulated progressively throughout development. Intermediate type II cells showed more prominent eggshell globule clusters and increased lipid accumulation, indicating further cellular differentiation. Mature vitelline cells contained large, well-developed eggshell globule clusters and abundant lipid droplets, representing the most advanced stage of vitelline cell differentiation. In contrast, glycogen particles and glycan vesicles were not observed at any developmental stage examined. Nurse cells exhibited extensive apical invaginations and close associations with adjacent developing vitelline cells. Gap junctions were observed between nurse cells and developing vitelline cells, suggesting intercellular communication and functional interactions during vitellogenesis. The coordinated development of eggshell globules and lipid inclusions, together with the close association between nurse cells and developing vitelline cells, indicates that vitelline cell differentiation is an organized process involving interactions between developing and supporting cells.

Conclusions: This study provides detailed histological and ultrastructural information on vitelline cell differentiation in *E. pancreaticum*. Vitellogenesis was characterized by the progressive formation and enlargement of eggshell globule clusters and the accumulation of lipid droplets, whereas glycogen particles and glycan vesicles were absent throughout the developmental sequence. The extensive apical invaginations and gap junctions observed between nurse cells and developing vitelline cells further indicate close structural and functional interactions during vitellogenesis. The distinctive combination of eggshell globule development, lipid accumulation, and nurse cell association may be related to the reproductive strategy of this embryonated egg-producing trematode. These findings contribute to the understanding of reproductive biology in *E. pancreaticum* and provide useful ultrastructural information for comparative studies of vitellogenesis and eggshell formation among digenetic trematodes.

Keywords: vitelline gland; trematode; eggshell formation; *Erytrema pancreaticum*

Introduction

Successful reproduction is essential for the survival, transmission, and life cycle completion of digenean trematodes. Egg production depends on the coordinated activities of the female reproductive system, particularly the vitelline glands, which produce vitelline cells responsible for eggshell formation, nutrient provision for embryonic development, and successful miracidium hatching (Rao, 1958; Irwin & Threadgold, 1972; Awad & Probert, 1990). Consequently, understanding the organization and differentiation of vitelline cells is fundamental to elucidating trematode reproductive biology and the developmental strategies of parasitic flatworms. Vitelline cells undergo a highly regulated process of differentiation during which they synthesize and accumulate cytoplasmic inclusions, including eggshell globules, lipid droplets, glycogen particles, and glycan vesicles. These inclusions contribute not only to eggshell assembly but also to the nutritional requirements of developing embryos. Ultrastructural studies have demonstrated that the composition and abundance of these inclusions vary among trematode species and are closely associated with their reproductive strategies (Erasmus, 1975; Threadgold, 1982; Świdorski *et al.*, 2019; Ualiyeva *et al.*, 2022). Such variations suggest that vitellogenesis may represent an adaptive process reflecting differences in embryonic development and egg maturation among digenean trematodes.

Most ultrastructural investigations of vitelline cells have been conducted in trematodes producing unembryonated eggs, including *Fasciola* spp. and *Schistosoma* spp., in which embryonic development occurs primarily after oviposition. In these species, vitelline cells provide both eggshell precursors and metabolic reserves, particularly glycogen and lipid, to support post-oviposition embryogenesis (Erasmus, 1975; Threadgold, 1982; Świdorski *et al.*, 2019; Ualiyeva *et al.*, 2022). In contrast, trematodes producing embryonated eggs complete miracidial development within the uterus before egg deposition, indicating that the functional requirements and storage products of vitelline cells may differ substantially from those of species producing unembryonated eggs. However, ultrastructural information on vitellogenesis in trematodes with this reproductive strategy remains limited. *E. pancreaticum* is a pancreatic fluke parasitizing cattle and other ruminants and is widely distributed throughout Asia. Unlike many digenean trematodes, it produces embryonated eggs containing fully developed miracidia at the time of oviposition. Despite its veterinary importance, the histology and ultrastructure of its vitelline gland have not previously been described. Consequently, it remains unknown whether vitelline cell differentiation, cytoplasmic inclusions, and nurse cell interactions exhibit structural adaptations associated with embryonated egg production.

Therefore, the present study investigated the histology and ultrastructure of the vitelline gland of *E. pancreaticum* using light microscopy and transmission electron microscopy. Particular emphasis was placed on characterizing the sequential stages of vitelline cell differentiation, describing the ultrastructural changes

associated with vitellogenesis, and examining the structural relationship between developing vitelline cells and surrounding nurse cells. The findings provide the first detailed ultrastructural description of vitellogenesis in *E. pancreaticum*, expand current understanding of reproductive adaptations in trematodes producing embryonated eggs, and establish a foundation for future comparative studies of vitellogenesis among digenean trematodes.

Methodology

Parasite Collection and Fixation

Five living adult *E. pancreaticum* specimens were collected from the pancreatic ducts of naturally infected cattle slaughtered at local licensed abattoirs in Pathum Thani Province, Thailand. Worms were rinsed several times in normal saline to remove host tissues and debris before fixation. Specimens were fixed in a solution containing 4% glutaraldehyde and 2% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS; pH 7.4) on ice for 4 h. After primary fixation, samples were washed three times in ice cooled PBS and stored at 4 °C until further processing.

Tissue Processing, Sectioning, Staining, and Microscopic Examination

Vitelline gland regions were carefully dissected from fixed specimens and cut into approximately 1 mm³ tissue blocks. Samples were post-fixed in 1% osmium tetroxide in 0.1 M phosphate-buffered saline (PBS) for 2 h, rinsed in PBS, dehydrated through a graded ethanol series, cleared in propylene oxide, infiltrated with a mixture of propylene oxide and Araldite 502 resin, and finally embedded in Araldite 502 epoxy resin followed by heat polymerization.

Semithin sections (0.5–1.0 µm thick) were cut using an ultramicrotome (Leica RM2125RTS), mounted on glass slides, dried on slide warmer, stained with 1% aqueous methylene blue, and examined under a light microscope (Olympus BX51). These sections were used to evaluate the general histological organization of the vitelline follicles, identify the distribution of developing vitelline cells, and select representative regions for subsequent ultrastructural examination.

Ultrathin sections (60–90 nm thick) were cut from selected regions of the resin-embedded tissue blocks, mounted on copper grids, stained with saturated alcoholic uranyl acetate followed by Reynolds' lead citrate, and examined using a transmission electron microscope (FEI. Tecnai 20) operated at 80 kV. Digital micrographs were acquired for ultrastructural analysis.

Ultrastructural Analysis and Classification of Vitelline Cells

Ultrastructural observations were performed on representative vitelline follicles obtained from all five adult worms. Vitelline cells were classified into four developmental stages (immature, intermediate type I,

intermediate type II, and mature) according to previously established ultrastructural criteria for trematode vitellogenesis (Erasmus, 1975), including cell size, nuclear morphology, cytoplasmic differentiation, and the presence, size, and distribution of eggshell globule clusters and lipid droplets.

Dimensions of cell and cellular structures were determined from digital transmission electron micrographs using Adobe Photoshop (Adobe Inc., San Jose, CA, USA). Measurements were calibrated against the scale bar provided on each micrograph and are reported as approximate values to describe the typical ultrastructural characteristics of each developmental stage rather than as quantitative morphometric data.

Ethics Statement

Adult *E. pancreaticum* specimens were obtained from cattle slaughtered for commercial food production at licensed abattoirs. No animals were euthanized specifically for this study. Sample collection was conducted between 2007 and 2008, prior to the implementation of the Animal for Scientific Purposes Act B.E. 2558 (2015) in Thailand. Therefore, institutional ethical approval was not required at the time of sample collection. This study complies with current ethical principles regarding the use of animal materials obtained from commercial sources.

Results

Organization of the Vitelline Gland

The vitelline gland of *E. pancreaticum* consisted of numerous vitelline follicles distributed bilaterally along the lateral regions of the body and embedded among the parenchymal cells (Fig. 1A). Each follicle was enclosed by a continuous epithelial layer of nurse cells supported by a basement membrane and basal lamina (Fig. 1B–D). The lumen of each follicle contained numerous developing vitelline cells at different stages of differentiation, arranged from the follicular periphery toward the central region (Fig. 1B–D). Based on their ultrastructural characteristics and cytoplasmic inclusions, developing vitelline cells were classified into four developmental stages: immature vitelline cells, intermediate vitelline cells type I, intermediate vitelline cells type II, and mature vitelline cells (Fig. 1B–D).

Immature Vitelline Cells: Undifferentiated Stage

Immature vitelline cells were located at the periphery of the vitelline follicle and were closely associated with the deep apical invaginations of nurse cells (Fig. 2A–C). These cells were the smallest among all developmental stages, measuring approximately 5–6 µm in diameter. Each immature vitelline cell contained a single nucleus measuring approximately 3.7 × 4.8 µm with small blocks of heterochromatin (approximately 0.4 × 1.0 µm) (Fig. 2A–C). The cytoplasm was poorly differentiated and contained predominantly free ribosomes

(Fig. 2C). At later stages of immaturity, mitochondria, tubular rough endoplasmic reticulum, and small Golgi complexes became evident within the cytoplasm (Fig. 2C).

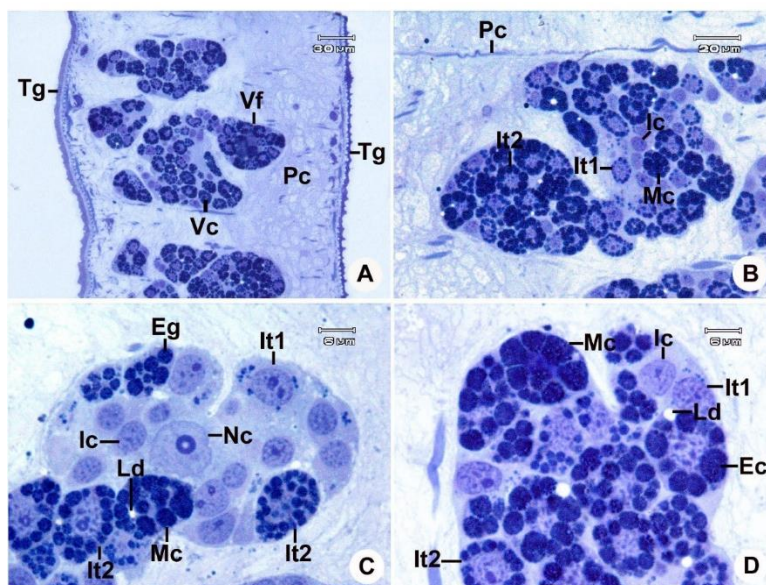


Figure 1 Light micrographs of the vitelline follicle of *E. pancreaticum*. (A) Low-, (B) medium-, and (C–D) high-magnification micrographs of semithin sections stained with methylene blue showing the overall organization of the vitelline follicle, including the follicular wall lined by nurse cells and developing vitelline cells at different stages; Vf - vitelline follicle; Pc - parenchymal cells; Vc - vitelline cells; Tg – tegument; Ic - immature cell; It1 - intermediate type I; It2 - intermediate type II; Ec - eggshell globule clusters; Ld - lipid droplet; Mc - mature cell; nurse cell (Nc).

Intermediate Vitelline Cells Type I

Intermediate vitelline cells type I were located in the intermediate region of the follicle and occupied the extracellular spaces formed by the apical invaginations of nurse cells (Fig. 2D–F). These cells measured approximately 7–8 μm in diameter. Their nuclei contained increased amounts of heterochromatin, with heterochromatin blocks measuring approximately 0.3–0.9 μm (Fig. 2D–F). The cytoplasm contained well-developed rough endoplasmic reticulum, numerous mitochondria, and prominent Golgi complexes (Fig. 2E, F). Small electron-dense eggshell globules measuring approximately 0.2–0.3 μm were observed within the Golgi cisternae (Fig. 2E). During the later phase of this stage, small eggshell globule clusters measuring approximately 1–2 μm and enclosed by Golgi-derived membranes were present in the cytoplasm (Fig. 2D, F).

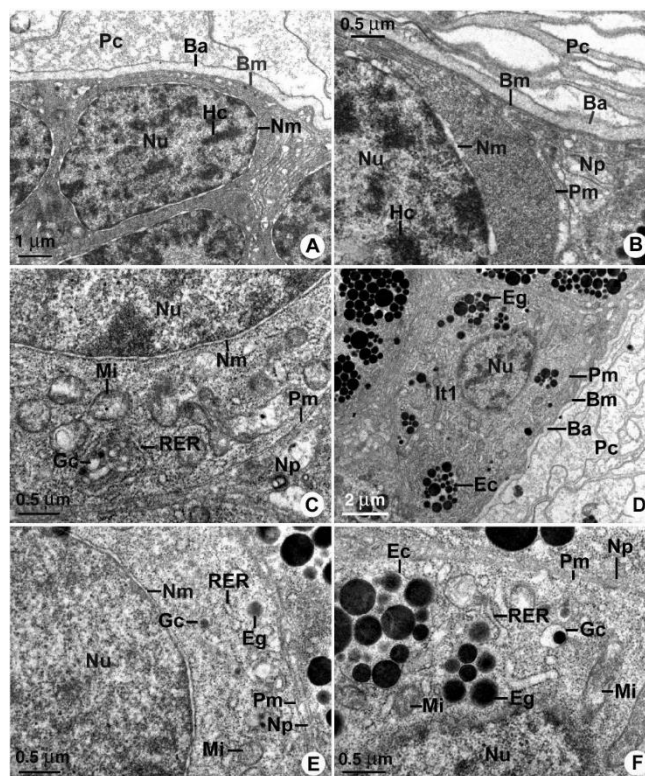


Figure 2 Transmission electron micrographs of developing vitelline cells. (A–C) Low (A), medium (B), and high (C) magnification of early immature vitelline cells showing a nucleus (Nu) predominantly composed of heterochromatin (Hc), with sparse cytoplasm and organelles, surrounded by nurse cell processes (Np); Bm – basement membrane; Ba – basal lamina; Pm – plasma membrane; Pc – parenchymal cell; Nm – nuclear membrane; RER – rough endoplasmic reticulum; Mi – mitochondria; Gc – Golgi complex. (D–F) Low (D) and high (E, F) magnification of intermediate type I (It1) vitelline cells showing small eggshell globules (Eg) and eggshell globule clusters (Ec) within the cytoplasm; Pc – parenchymal cell; Ba – basal lamina; Pm – plasma membrane; Bm – basement membrane; RER – rough endoplasmic reticulum; Mi – mitochondria; Gc – Golgi complex; Np – nurse cell process; Pm – plasma membrane; Nu – nucleus; Nm – nuclear membrane.

Intermediate Vitelline Cells Type II

Intermediate vitelline cells type II measured approximately 10–12 μm in diameter and were located closer to the apical region of the nurse cells (Fig. 3A–C). Their nuclei measured approximately $4.4 \times 6.0 \mu\text{m}$ and contained large heterochromatin blocks measuring approximately $0.7 \times 1.2 \mu\text{m}$ (Fig. 3A–C). The cytoplasm

was occupied predominantly by large eggshell globule clusters measuring approximately 3–4 μm (Fig. 3A–C). Surrounding Golgi membranes were no longer observed around the globule clusters (Fig. 3B, C). Small lipid droplets measuring approximately 1.5–2.0 μm were present among the eggshell globule clusters (Fig. 3B).

Mature Vitelline Cells

Mature vitelline cells were the largest cells, measuring approximately 13–15 μm in diameter, and were located predominantly in the central region of the vitelline follicle (Fig. 3D–F). The nucleus was characteristically star-shaped, measuring approximately 5.0–6.3 μm , and contained abundant heterochromatin blocks measuring approximately $0.6 \times 1.3 \mu\text{m}$ (Fig. 3D–F). The cytoplasm was densely packed with large eggshell globule clusters measuring approximately 5–6 μm , which lacked surrounding membranes and occupied most of the cytoplasmic volume (Fig. 3D–F). Large lipid droplets approximately 3 μm in diameter were interspersed among the eggshell globule clusters (Fig. 3D–F). Only a few mitochondria and tubular rough endoplasmic reticulum were observed, mainly between the globule clusters and around the nucleus (Fig. 3E, F). Glycogen particles and glycan vesicles were not observed at any stage of vitelline cell development.

Nurse (Supporting) Cells

Nurse cells formed a continuous epithelial layer surrounding each vitelline follicle (Fig. 4A). Their basal plasma membrane was closely associated with a smooth basement membrane and a thick basal lamina without basal infoldings (Fig. 4B). In some regions, the basal lamina was attenuated or absent, allowing direct contact between nurse cells and adjacent parenchymal cells (Fig. 4B). The apical plasma membrane exhibited numerous deep invaginations extending toward the basal region and frequently reaching the perinuclear area (Fig. 4A–D). Some apical invaginations exhibited localized membrane thickening, forming intracytoplasmic junctions (Fig. 4C, D). Developing vitelline cells were frequently located within the extracellular spaces formed by these apical invaginations and were connected to nurse cells by gap junctions (Fig. 4E, F). The nucleus of nurse cells measured approximately $3.6 \times 4.0 \mu\text{m}$ and consisted predominantly of euchromatin with a prominent nucleolus (Fig. 4A, C). A distinct nuclear envelope and thick nuclear lamina surrounded the nucleus (Fig. 4C, D). The cytoplasm contained abundant smooth endoplasmic reticulum distributed throughout the cell (Fig. 4A–D).

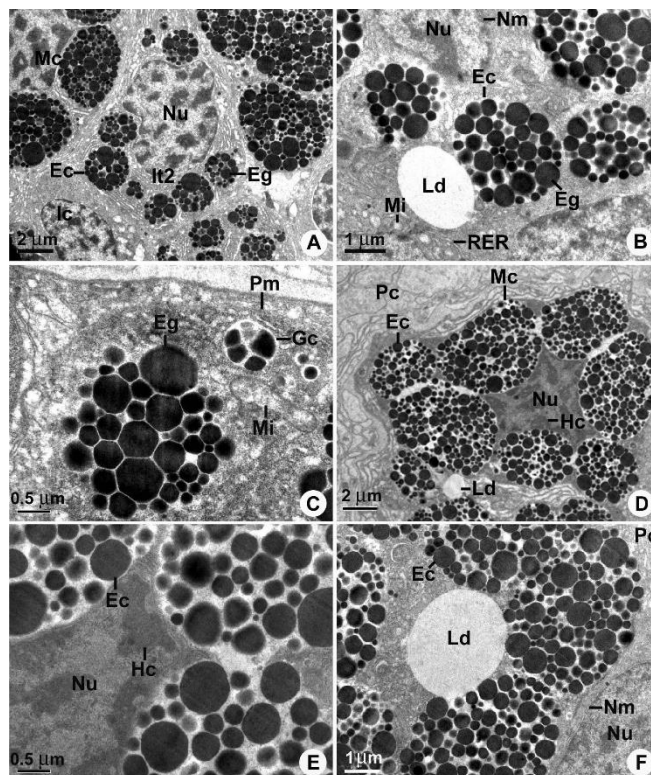


Figure 3 Transmission electron micrographs of advanced vitelline cell development of *E. pancreaticum*.

(A–C) Low (A), medium (B), and high (C) magnification of intermediate type II (It2) vitelline cells showing small to medium eggshell globules (Eg) aggregated into eggshell globule clusters (Ec), together with lipid droplets (Ld), rough endoplasmic reticulum (RER), and mitochondria (Mi). The Golgi complex contains individual and grouped globules, while large, non-membrane-bound clusters (Ec) are dispersed throughout the cytoplasm; Lc – immature cell; Mc – mature cell; Nu – nucleus; Pm – plasma membrane. (D–F) Low (D) and high (E, F) magnification of mature vitelline cells (Mc) showing numerous eggshell globules aggregated into large clusters (Ec) distributed throughout the cytoplasm. RER and mitochondria are interspersed among the clusters and concentrated around the nucleus; Ld – lipid droplet; Nu – nucleus; Nm – nuclear membrane; Hc – heterochromatin; Pc – parenchymal cell.

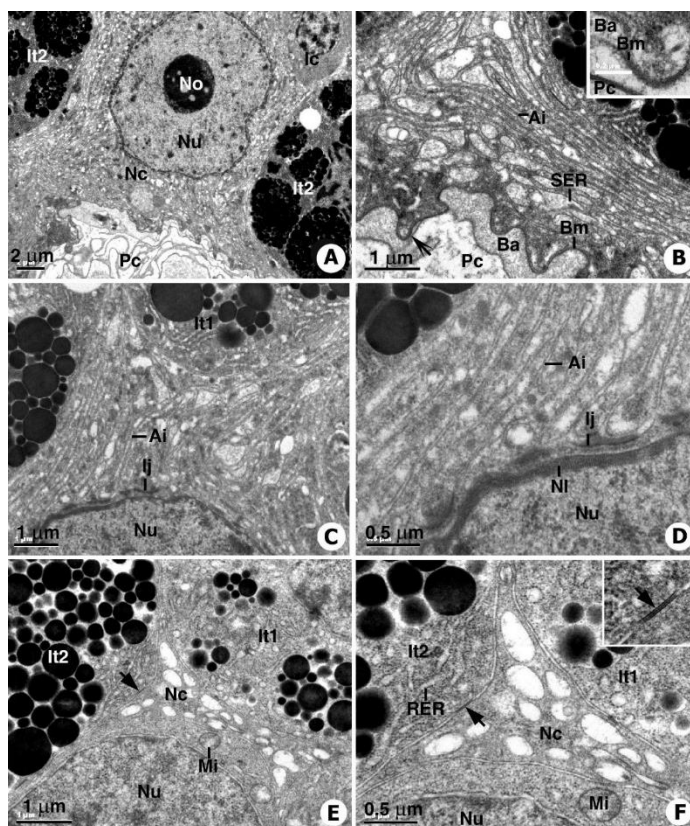


Figure 4 Transmission electron micrographs of nurse cells (Nc) in vitelline follicle of *E. pancreaticum*.

(A) Low magnification showing the nucleus (Nu) with a prominent nucleolus (No), surrounded by immature (Ic), intermediate type II (It2), and parenchymal cells (Pc). (B) Medium magnification of the basal region showing the basal plasma membrane attached to the basement membrane (Bm) and basal lamina (Ba); focal thinning permits direct contact with parenchymal cells (Pc) (arrows, inset). The cytoplasm contains abundant smooth endoplasmic reticulum (SER) and numerous apical invaginations (Ai). (C, D) Medium (C) and high (D) magnification of the cytoplasm–nucleus interface showing thickened nuclear lamina (NI), where apical invaginations (Ai) extend toward the nucleus; It1 – intermediate type I. (E, F) Medium (E) and high (F) magnification of the apical region showing gap junctions (arrows) between nurse cells and adjacent vitelline cells; Mi – mitochondria; RER – rough endoplasmic reticulum; Nu – nucleus; It1 – intermediate type I; It2 – intermediate type II.

Table 1 The table summarizes the key ultrastructural characteristics of vitelline cells in *E. pancreaticum* across four developmental stages. Measurements are based on calibrated transmission electron micrographs and are presented as approximate values. Only major diagnostic features relevant to vitelline cell differentiation, including heterochromatin distribution, eggshell globule formation, and lipid accumulation, are included.

Feature	Immature	Intermediate type I	Intermediate type II	Mature cell
Cell size (µm)	5–6	7–8	10–12	13–15
Nucleus morphology	Large, round–oval	Round–oval	Oval	Star-shaped
Nuclear size (µm)	3.7 × 4.8	~4.0 × 5.2	4.4 × 6.0	5.0–6.3
Heterochromatin	Few small blocks	Moderately blocks	Large blocks	Abundant
Eggshell globules	Absent	Present	Present	Present
Eggshell globule clusters	Absent	1–2 µm	3–4 µm	5–6 µm
Lipid droplets	Absent	Absent	Present (1–2 µm)	Present (3–4 µm)
Glycogen / glycan vesicles	Absent	Absent	Absent	Absent

Discussion

Organization of the Vitelline Gland and Nurse Cell Function

The vitelline gland of *E. pancreaticum* consists of numerous follicles arranged bilaterally along the lateral regions of the body, a pattern commonly reported in digenean trematodes (Rao, 1958; Erasmus, 1975; Threadgold, 1982; Świdorski *et al.*, 2019; Ualiyeva *et.al.* 2022). Within each follicle, developing vitelline cells are organized from the peripheral region toward the center according to their developmental stage. A similar maturation gradient has been described in *Fasciola hepatica*, *F. gigantica*, and *Schistosoma mansoni*, suggesting that this arrangement is a conserved feature of vitellogenesis in digenean trematodes (Erasmus, 1975; Threadgold, 1982; Meepool *et al.*, 2006).

The present study also demonstrated that each vitelline follicle is enclosed by a continuous layer of nurse cells. Their extensive apical membrane invaginations and close association with developing vitelline cells may facilitate metabolite exchange during vitellogenesis, as previously proposed in other trematodes (Irwin & Threadgold, 1970; Erasmus, 1975). In addition, the presence of gap junctions between nurse cells and developing vitelline cells suggests intercellular communication during vitelline cell differentiation (Threadgold, 1982). The abundant smooth endoplasmic reticulum observed within nurse cells may be associated with

metabolite transport and lipid metabolism rather than direct synthesis of eggshell material. Furthermore, focal attenuation of the basal lamina may permit exchange between nurse cells and the surrounding parenchyma, consistent with previous observations in digenean trematodes (Awad & Probert, 1990).

Vitelline Cell Differentiation and Eggshell Formation

Four developmental stages of vitelline cells were recognized in *E. pancreaticum*: immature, intermediate type I, intermediate type II, and mature cells. This developmental sequence generally resembles that described in *F. hepatica*, *F. gigantica*, *Paragonimus skrjabini*, *Opisthorchis viverrini*, *Azygia lucii*, and *S. mansoni*, indicating that the overall process of vitellogenesis is largely conserved among digenean trematodes (Threadgold, 1982; Pang *et al.*, 1991; Meepool *et al.*, 2006; Ualiyeva *et al.*, 2022; Ruangsittichai *et al.*, 2006).

Immature vitelline cells were characterized by sparse cytoplasmic organelles and numerous free ribosomes, whereas intermediate type I cells exhibited well-developed rough endoplasmic reticulum and Golgi complexes together with the first appearance of electron-dense eggshell globules. These observations are consistent with active synthesis and packaging of eggshell precursor material during this stage (Irwin & Threadgold, 1972; Threadgold, 1982). Eggshell globules subsequently aggregated into progressively larger clusters during intermediate type II and mature stages, while Golgi membranes gradually disappeared. Similar ultrastructural changes have been reported in several trematode species and are considered characteristic features of vitelline cell maturation (Meepool *et al.*, 2006; Świdorski *et al.*, 2019).

Lipid droplets first appeared in intermediate type II cells and became prominent in mature vitelline cells. Lipid accumulation has also been reported in *S. mansoni* and other digeneans and is generally regarded as an important energy reserve for embryonic development (Erasmus, 1975; Threadgold, 1982). The simultaneous presence of abundant lipid droplets and eggshell globule clusters in mature vitelline cells indicates that these cells retain both structural and nutritional components before incorporation into the developing egg.

Functional Significance of the Absence of Glycogen and Glycan Vesicles

A notable finding of the present study was the absence of glycogen particles and glycan vesicles throughout all stages of vitelline cell differentiation. In contrast, these inclusions have been reported in several trematodes producing unembryonated eggs, including *Fasciola hepatica*, *F. gigantica*, *Paragonimus skrjabini*, *Echinostoma caproni* and *Cainocreadium labracis* (Threadgold, 1982; Meepool *et al.*, 2006; Pang *et al.*, 1991; Schmidt, 1998; Świdorski *et al.*, 2024). Interestingly, glycogen has also been reported in the vitelline cells of the embryonated egg-producing trematode *A. lucii* (Ualiyeva *et al.*, 2022), indicating that the presence or absence of glycogen is not determined solely by the embryonated egg strategy.

Unlike *A. lucij*, *E. pancreaticum* lacked both glycogen particles and glycan vesicles throughout vitelline cell differentiation. This difference suggests that vitelline cell composition may also reflect species-specific adaptations associated with reproductive biology, embryonic development, or hatching mechanisms. In *E. pancreaticum*, embryonic development is completed before oviposition, and hatching occurs only after the eggs are ingested by the snail intermediate host. These characteristics may reduce the requirement for carbohydrate storage within vitelline cells. Similarly, the absence of glycan vesicles may be related to differences in the hatching mechanism compared with species such as *F. hepatica* and *F. gigantica*, in which glycan vesicles have been proposed to contribute to hydrostatic pressure during miracidial hatching (Seed & Bennett, 1978; Wells & Cordingley, 1991; Meepool *et al.*, 2006). Although these interpretations require further experimental confirmation, the ultrastructural differences observed in *E. pancreaticum* suggest that vitelline cell composition is influenced by multiple biological factors rather than by embryonation status alone.

Conclusions

This study provides the first histological and ultrastructural description of the vitelline gland in *E. pancreaticum*. Four developmental stages of vitelline cells were identified, demonstrating the progressive formation of eggshell globule clusters and the accumulation of lipid droplets during maturation. The absence of glycogen particles and glycan vesicles distinguishes *E. pancreaticum* from several trematodes. These findings expand current knowledge of vitellogenesis in digenean trematodes and provide a basis for future comparative studies.

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