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Ultrastructural organization of the oviducts of the mayfly *Habrophlebia eldae* (Ephemeroptera, Leptophlebiidae)

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ABSTRACT

The genital system of the mayfly *Habrophlebia eldae* consists of paired ovaries formed by ovarioles of the panoistic type. These ovarioles are connected to lateral oviducts that join to form a common duct. By transmission electron microscopy (TEM), the wall of the oviducts and of the common duct consists of an inner layer of epithelial cells and an outer muscular sheet. In the anterior region of the lateral oviducts the epithelial cells present an irregularly-shaped microvillar border. In the posterior region the cells are convered by epicuticle. In the common duct thin extensions protrude from the cuticle border of the epithelial cells towards the lumen. No accessory glands are present in the genital system of *H. eldae*. The organization of the lateral oviducts and common duct is discussed with an eye to the reproductive biology of leptophlebiids.

KEY WORDS: Ephemeroptera; Leptophlebiidae; *Habrophlebia eldae*; Female genital system; Ultrastructure.

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INTRODUCTION

Considerable attention has been paid to Ephemeroptera in an attempt to identify morphological characteristics useful for taxonomic purposes, but few studies have dealt with the development and organization of the mayfly reproductive system. Some histological data are given in classical monographs which report the basic type of the female reproductive system as having paired ovaries with panoistic ovarioles and oviducts (Brinck, 1957; Soldàn, 1979a). The genital area and openings are differently arranged within the mayfly genera to make up for the lack of specialized surroundings for reception and storage of sperm and for oviposition after mating (Grandi, 1947, 1955; Brinck, 1957). In particular, spermatozoa of leptophlebiids are immotile (Soldàn, 1979b) and a penis muscle ring, which pumps sperm into the female genital system, obviates sperm immotility (Grimm, 1985). How fertilization is achieved by these immotile sperm is still unknown; it might be facilitated by specialized structures of the female genital system. The present study is concerned with the fine organization of the oviducts in order to ascertain their role in facilitating fertilization.

MATERIAL AND METHODS

The reproductive apparatus of *Habrophlebia eldae* Jacob & Sartori, 1984 was dissected from young nymphs characterized by meso-and metathoracic wing-pads enveloping wings with readily visible veins (Gaino, 1987).

For ultrastructural investigation, selected material was fixed for 1 h in 5% glutaraldehyde-4% formaldehyde in 0.1 M cacodylate buffer at pH 7.2 (Karnovsky, 1965), rinsed in buffer and postfixed for 1 h in osmium tetroxide in 0.1 M cacodylate buffer at pH 7.2. After dehydration in a graded series of ethanol and in propylene oxide, the material was embedded in an Epon-Araldite mixture. Thin sections were cut on a Reichert ultramicrotome, mounted on formvar-coated copper grids and examined with Philips 300 and 400 transmission electron microscopes (TEM) after staining in aqueous uranyl acetate and lead citrate.

RESULTS

The female reproductive apparatus of *Habrophlebia eldae*, as shown schematically in Figure 1, consists of paired ovaries and oviducts, which join to form a common duct. Both the lateral oviducts and the common duct are characterized by an inner layer of epithelial cells lining the lumen and an outer layer of muscle cells. Each lateral oviduct presents an anterior and a posterior region that are differently organized (Fig. 1). In the anterior region the epithelial cells present numerous folds at their apical part (Fig. 2), resulting in an irregularly-shaped microvillar border containing electron-dense vesicles (Fig. 3). A narrow band of muscle cells with hardly distinguishable nuclei envelops the epithelium (Fig. 2).

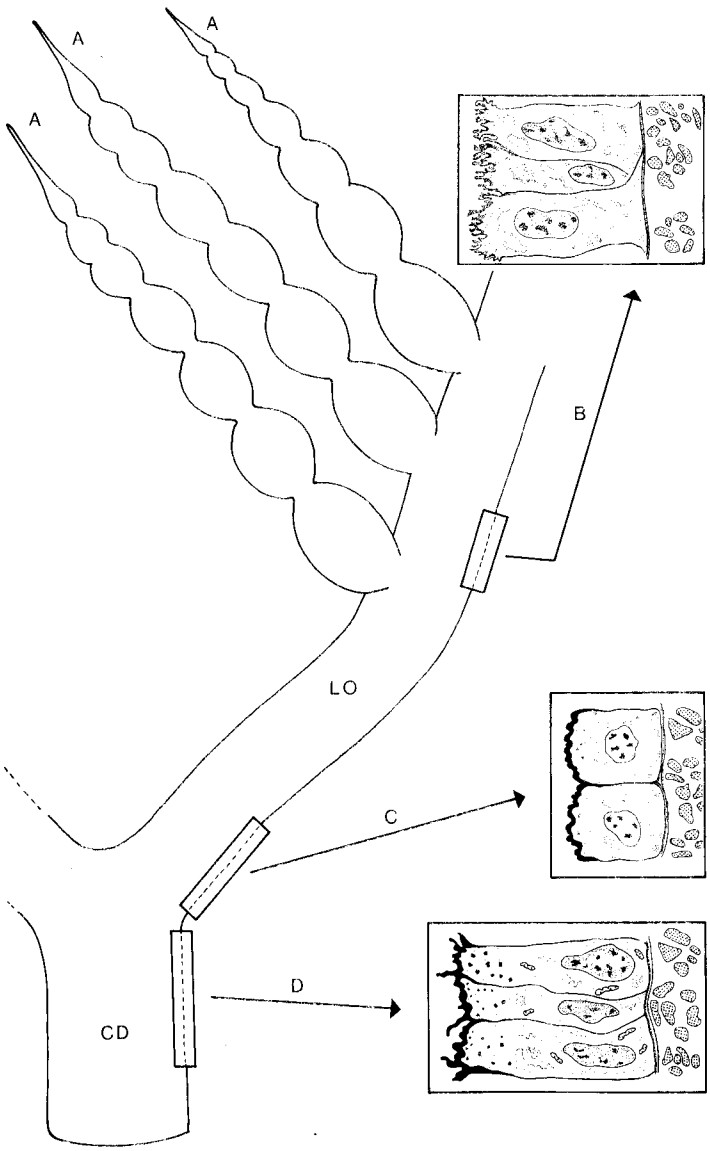


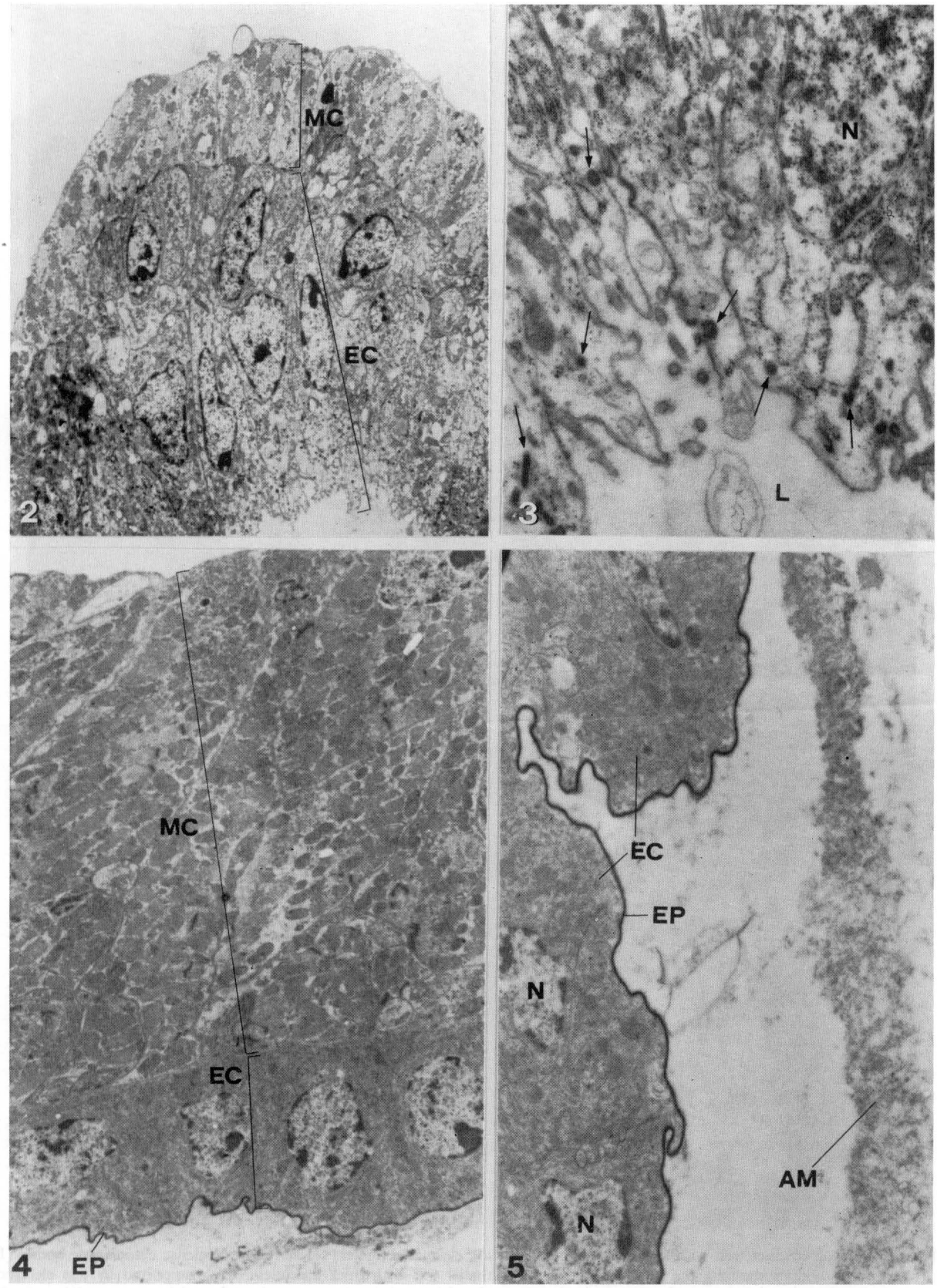
Fig. 1 - Schematic drawing of the female reproductive system of *Habrophlebia eldae* showing the arrangement of the panoistic ovarioles (A) merging with the lateral oviducts (LO). Both lateral oviducts join in a common duct (CD). Ducts differ in cell structure: B, region with cells characterized by an irregularly-shaped microvillar border; C, region with cuboidal epithelial cells covered by epicuticle; D, region with columnar epithelial cells covered by epicuticle that forms thin villous projections.

In the posterior region a thin layer of cuboidal cells with large, centrally located nuclei are seen. The luminal border of these cells is covered by an epicuticle (Fig. 4). The epithelial surface is occasionally folded to produce irregularly-shaped protrusions that extend towards the oviduct lumen (Fig. 5). Strands of adhesive fibrous material are present in the lumen or close to the epicuticle border (Fig. 5). Unlike that of the anterior region, the outer muscular sheet is very thick (Fig. 4).

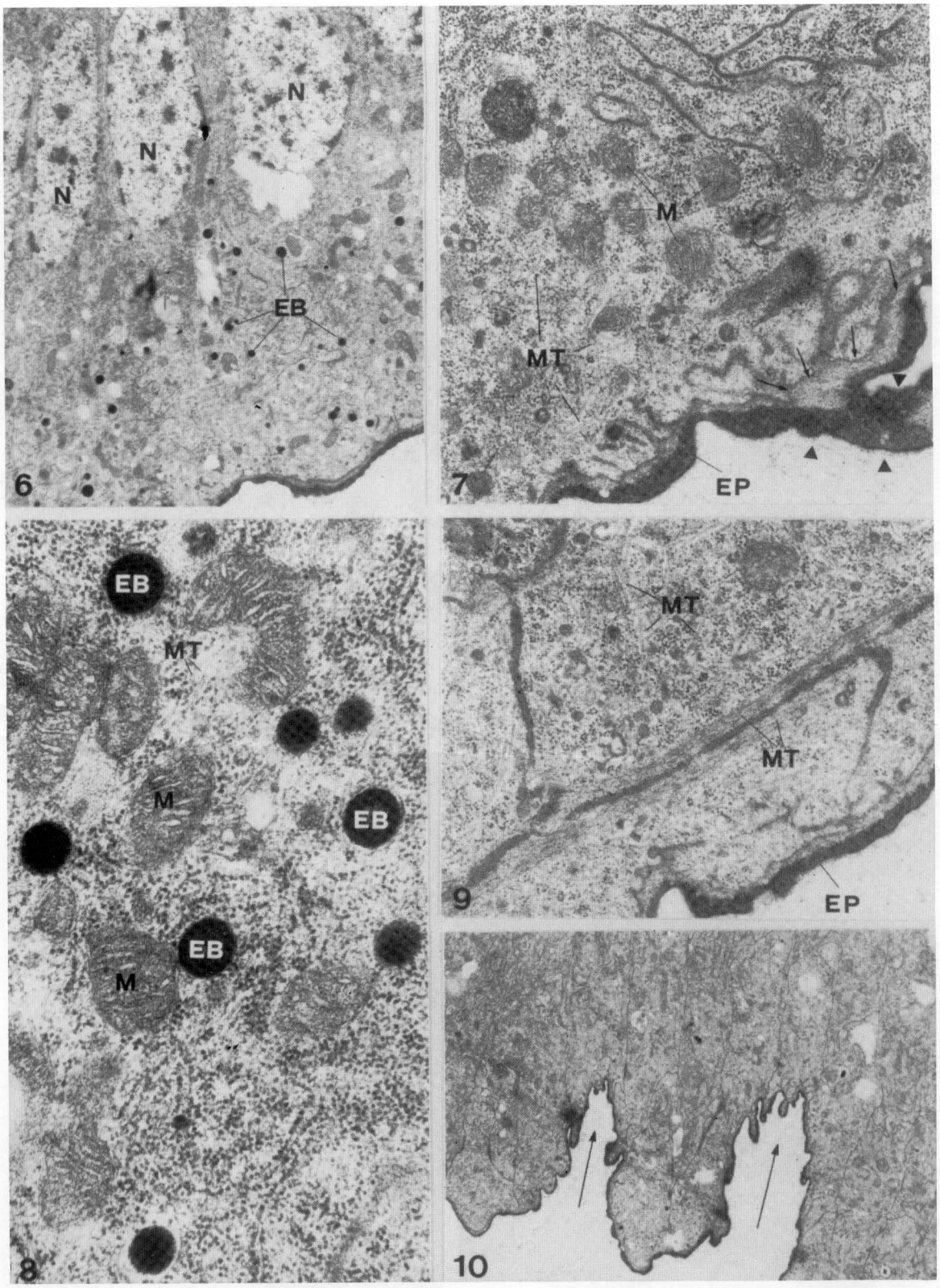
Like the posterior region of the oviducts, the common duct also consists of a thick outer layer of muscle cells and an inner layer of epithelium (Fig. 1). The epithelial cells are columnar, with elongated nuclei located at their bases (Fig. 6). They are covered by an epicuticle that borders the common oviduct walls (Figs 7, 9, 10). The epicuticle is closely associated with a less electron-dense layer representing the exocuticle (Fig. 7). The cytoplasm is abundant in mitochondria, microtubules, free ribosomes and scattered electron-dense bodies of about 0,2 μm in diameter (Fig. 8). The cells are extensively linked by junctional complexes associated with closely packed microtubule bundles (Fig. 9). Epithelium folds (Fig. 10) form longitudinal ridges along the luminal walls and show villus-like protrusions towards the lumen (Fig. 10). In some areas the protrusions exhibit an exocuticle core surrounded by epicuticle (Fig. 7).

No accessory reproductive glands are associated with the common oviduct.

In the adult, the common duct extends in a tubular portion (i.e., the ovipositor or egg guide) which extends as far as the middle of the 8th abdominal segment and serves for both sperm reception and oviposition. There is a single gonopore for oviposition after mating.



Figs 2-5 - 2. Lateral oviduct wall in the anterior region with the inner epithelial cells (EC) and the outer layer of muscle cells (MC). (× 5 800). 3. The epithelial cells show a luminal side with vesicles (arrows) close to the lumen (L); epithelial cell nucleus (N). (× 31 000). 4. Lateral oviduct wall in the posterior region showing epithelial cells (EC) delimited by an epicuticle (EP) and a thick layer of muscle cells (MC). (× 6 400). 5. Detail of the posterior region of the lateral oviduct. A strand of adhesive material (AM) is located above the epithelium covered by epicuticle (EP). Epithelial cells (EC); epithelial cell nucleus (N). (× 12 000).



Figs 6-10 - 6. Common oviduct wall with the epithelial cells showing elongated nuclei (N) and electron-dense cytoplasmic bodies (EB). (× 7 600). 7. Common oviduct showing the luminal side of an epithelial cell with thick epicuticle (EP) supported by less electron-dense exocuticle (arrows). Note the basal part of a thin projection (arrowheads) consisting in outer epicuticle and inner exocuticle; mitochondria (M), microtubules (MT). (× 31 000). 8. Detail of the cytoplasm content of a cell delimiting the common oviduct wall, abundant in mitochondria (M) microtubules (MT) and scattered electron dense bodies (EB). (× 50 000). 9. Detail of the junctional area between adjacent epithelial cells of the common oviduct. Abundant bundles of microtubules (MT) are visible, particularly at the junctional level; epicuticle (EP). (× 31 000). 10. Folding of the common oviduct wall showing numerous thin microextensions (arrows) protruding towards the lumen. (× 6 800).

DISCUSSION

Our results outline the morphological differences in the various constituents of the female reproductive system of *Habrophlebia eldae*. In view of the previous anatomic studies on mayflies (Grandi, 1943, 1947, 1955; Brinck, 1957), the presence of a common oviduct is an unusual feature. An evolutionary trend may be traced in leptophlebiids from a more primitive organization characterized by gonopores which open directly to the exterior, such as in *Choroterpes*, to a more differentiated tubular ovipositor protruding outside, such as in *Habrophlebia*. This trend probably reflects the phylogeny of the ancient Leptophlebiidae family, the most diversified mayfly group in the world (Peters, 1980).

The architecture of the posterior region of the oviducts and of the common duct is consistent with the need to expand greatly during egg passage and accumulation. This function is possibly enhanced by folding of the epithelium and stretching of the underlying muscles. In addition, the wide muscle sheath enveloping the common duct of *H. eldae* may facilitate sperm/egg interaction after mating by peristaltic movements. A similar mechanism has been previously suggested for the female genital ducts of psicodids where muscle contraction obviates sperm immotility and allows fertilization (Burrini & Dallai, 1975). These observations support the view that sperm immotility is associated with the development of a wide muscle sheath around male and female genital ducts.

The secretion of the oviduct epithelium, previously documented in this species, is synthesized by the cells delimiting the apical region of the lateral oviducts (Gaino & Mazzini, 1989), which make up for the absence of accessory reproductive glands in Ephemeroptera (Brinck, 1957). The secretory activity of the female genital ducts has recently been suggested for egg coverings also in two representatives of Collembola (Larink & Bilinski, 1989) and has repeatedly been documented at ultrastructural level in several groups of insects (Dallai, 1967; Dallai & Melis, 1966-67; Longo *et al.*, 1982; Melis & Dallai, 1966-67; Sweeney *et al.*, 1968). In particular, in a coleopteran species the lateral oviducts are involved in the secretion of electron-dense substances except for their posterior portions, which are covered by epicuticle and do not perform any secretive function (Biemont *et al.*, 1981; Hamon *et al.*, 1982). In the common oviduct of *H. eldae*, electron-dense bodies are observed in the cytoplasm of the epithelial cells covered by intima. These data suggest the involvement of these cells in adhesive layer secretion, although no material has been observed to enter the lumen at this level. Adhesive layers, besides assisting the passage of the mature eggs and preserving them during storage in the female apparatus, could participate in the mechanism of fertilization. Indeed, the eggs of some Amphibia are enveloped by oviductal products (Yoshizaki, 1985). These include the jelly envelopes, which enhance fertilization by improving sperm penetration (McLaughlin & Humphries, 1978; Jego *et al.*, 1983).

In conclusion, the architecture of the distal region of the lateral oviducts and that of the common duct results in epithelium folds and in thick sheaths of muscle cells. This organization appears to facilitate: 1. egg accumulation by enlarging of the oviducts and stretching of the muscles; 2. immotile sperm/egg interaction by adhesive layer secretion and peristaltic muscle contraction.

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