Structural Changes of the Female Genital System during and after Feeding in Haemaphysalis longicornis (Acari: Ixodidae)

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Structural Changes of the Female Genital System during and after Feeding in *Haemaphysalis longicornis* (Acari: Ixodidae)

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Structural changes in the female genital system of *Haemaphysalis longicornis* during feeding, copulation and oviposition were observed in the light microscope. The genital system consists of the vestibular and cervical vagina, tubular accessory glands, connecting tube, receptaculum seminis, oviduct and ovary. As in *Hyalomma*, epithelial cells lining the vestibular cuticle become the lobular accessory glands during feeding, secretions from which coat the surface of passing eggs and may function as a waterproofing agent. However, it is clear from these observations that dramatic feeding-related changes occur also in the other organs constituting the system. The cervical vagina and connecting tube both have a similar structure, their developed muscles being involved in transfer of male germ cells and/or eggs. Secretions from the feeding-stimulated tubular accessory glands may be a coating agent applied to the egg surface or act as a lubricant for egg passage. Epithelial cells of the receptaculum seminis are probably involved in destruction of the inserted endospermatophore. In the fed females, just, after copulation the oviducal epithelium produces a secretion, which also may exert some influence upon the passing spermatozoa and eggs. Muscles surrounding the oviducts cause peristaltic contractions during the passage of spermatozoa and eggs. In the developed ovary, the oocyte protrudes into the haemocoel, and is attached to special funicle cells which arise from ovarian epithelium. Numerous spermatozoa present in the ovarian lumen during oviposition imply that fertilization occurs in the ovary.

INTRODUCTION

Copulation of ticks is completed by the insertion of the endospermatophore derived from the male into the female genital aperture. Spermatids transferred into the female undergo spermiogenesis during ascent in the female genital tract and become mature spermatozoa (Borut and Feldman-Muhsam, 1976; Wüest et al., 1978; El Said et al., 1981), which fertilize oocytes. Thousands of fertilized eggs pass back through the tract regularly, to be released from the genital aperture one by one. The organs which constitute the female genital system have characteristic features, contributing both to the ascent of male germ cells (spermatids and spermatozoa) and to the passage of eggs. In addition, there exist interesting interrelationships between the germ cells and tissues of the female tract (Brinton et *al., 1974).*

These organs change dramatically soon after a blood meal (El Shoura, 1989). To discover whether such feeding-related genital tract changes occur as well in other ticks, we have made a fundamental study of the interrelationship between germ cells and the

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female genital system before and after feeding, in *Haemaphysalis longicornis* in the light microscope, as reported here, and at the ultrastructural level (in preparation).

MATERIALS AND METHODS

Adult *Haemaphysalis longicornis* (bisexual strain) collected by dragging on pastures in Kuju Highland, Oita Prefecture, were allowed to feed by attaching to the ears of laboratory rabbits. Engorged females were maintained in a dark incubator at constant temperature and humidity (30°C, 90%RH). Unfed, feeding (5 days after attachment) and ovipositing (1 day after the onset of oviposition) females, were examined. After the

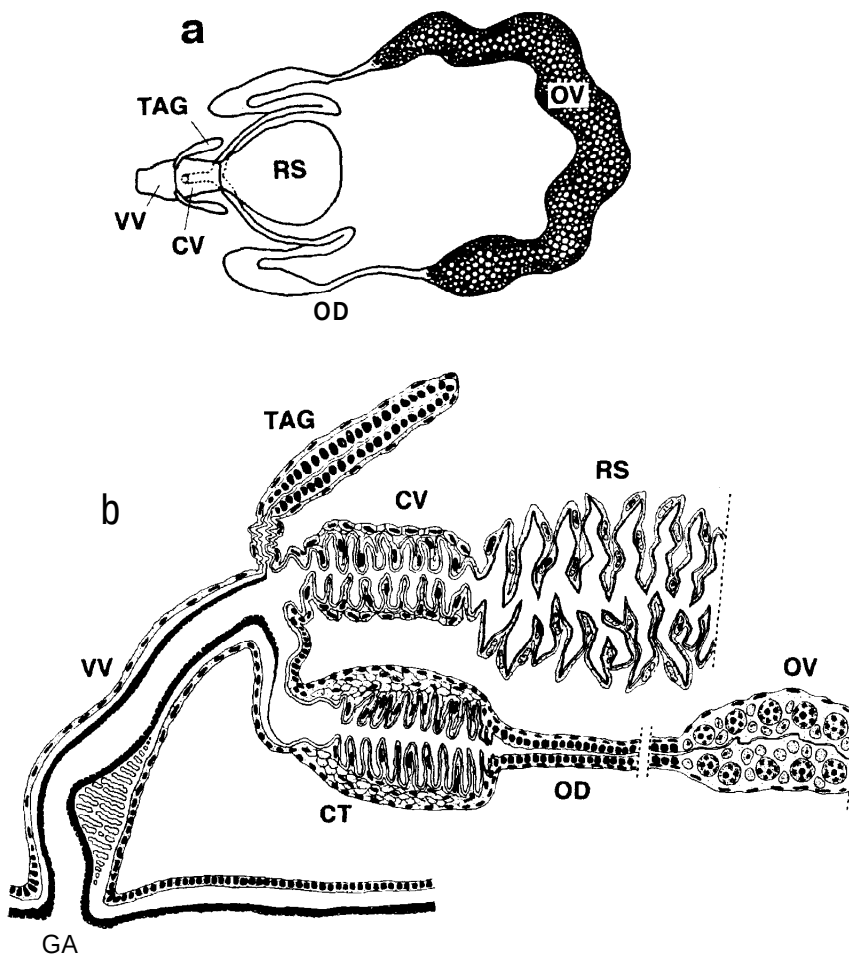


Fig. 1. Diagrams of the dorsal (a) and lateral (b) view of the genital system in the unfed female *Haemaphysalis longicornis*. CT, connecting tube; CV, cervical vagina; GA, genital aperture; OD, oviduct; OV, ovary; RS, receptaculum seminis; TAG, tubular accessory gland; W, vestibular vagina.

removal, the genital system was fixed with 3% glutaraldehyde in 0.1 M sodium cacodylate buffer (4°C, pH 7.2), washed thoroughly in the same buffer, and then dehydrated in an ethanol series and embedded in Kulzer Technobit 7100 metacrylate resin. Sections (~1.5 μm) were doubly stained with haematoxylin and eosin.

RESULTS

Beginning at the genital aperture, the female genital system of *Haemaphysalis longicornis* consists of the vestibular vagina, cervical vagina, paired tubular accessory glands, receptaculum seminis, connecting tube, oviducts and a looped ovary (Fig. 1). A pair of oviducts fuse into a single common oviduct just behind the connecting tube.

1. Vestibular vagina

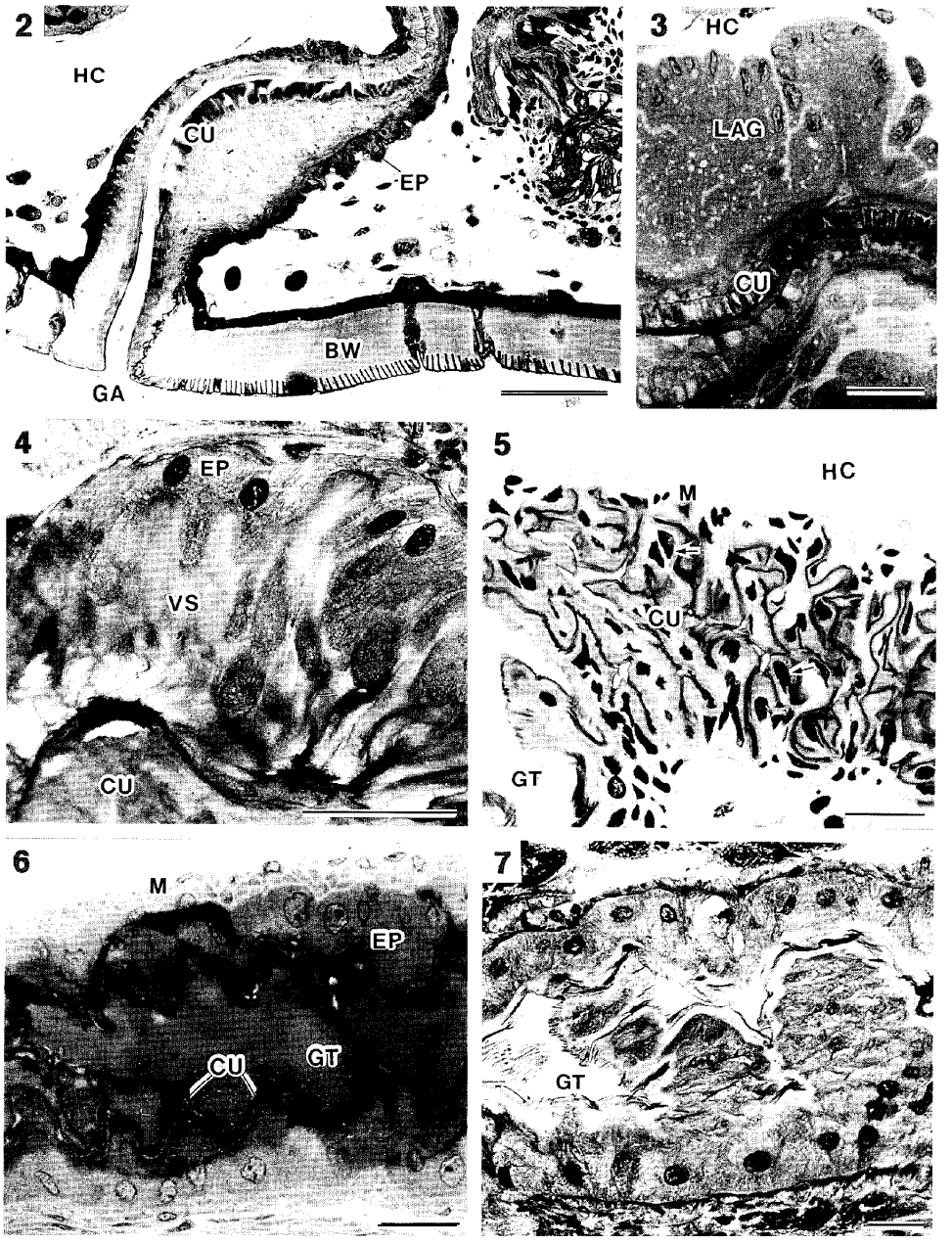
The vestibular vagina leads to the genital aperture and has a thick cuticle which continues along the ventral body wall (Fig. 2). A layer of squamous or cuboidal epithelium lines the vestibular cuticle in the unfed stage, then increases in volume during feeding, to become the lobular accessory glands (Fig. 3). Their epithelial cells have a nucleus situated in the basal region (haemocoelic side) and their cytoplasm contain numerous small vesicles. During oviposition, in the lobular accessory glands the apical region of the epithelium detaches from the cuticle and the large lumen so formed is called the vestibular sinus (Fig. 4). The sinus lumen is filled with eosinophilic secretion.

2. Cervical vagina

The cervical vagina has a folded thin cuticle lined with epithelial cells, externally surrounded by the muscle layer (Fig. 5). During feeding, the cuticle unfolds with longitudinal enlargement of the cervical vagina (Fig. 6). The lumen (genital tract) is filled with material derived from the endospermatophore inserted by the male at copulation. The cytoplasm of the epithelial cells increases in volume and the nucleus displays increased euchromatin. Muscles immediately external to the tract also develop during feeding and become stratified. During oviposition, no structural changes are observed in either the epithelium or the cuticle, but the muscle layers become thinner and the material in the lumen disappears (Fig. 7).

3. Receptaculum seminis

In the unfed stage, the large sac of the receptaculum seminis has a highly folded thin cuticle whose lumen is closed and invisible in the light microscope, and it has no muscle layer (Fig. 8). Simple sqamous epithelial cells which line the cuticle possess an oval nucleus (diam. ~5 μm) with increased euchromatin. After copulation, the receptaculum seminis containing the endospermatophore becomes distended and its folded cuticle almost flattens (Fig. 9). The epithelial cells with enlarged cytoplasm and a round nucleus actively secrete substances that stain strongly with haematoxylin. Numerous spermatids and granular structures are present in the spermatophore (Fig. 9). Later, at the time of oviposition, spermatids and spermatozoa have become invisible, residues of the endospermatophore can be seen in the lumen, and the cytoplasm of the epithelial cells



Figs. 2-4. Light micrographs of sagittal section through the vestibular vagina. Fig. 2. The unfed female, showing the thick cuticle (CU) leading to the ventral body wall (BW). An epithelial layer (EP) lines the cuticle. Scale bar = 50 pm. Fig. 3. The epithelium increases in volume during feeding, becoming the lobular accessory glands (LAG). Scale bar=10 μm. Fig. 4. The ovipositing female, showing the developed lobular accessory glands. Epithelial cells of the glands detach from the cuticle, and the vestibular sinus (VS) is formed between the epithelium (EP) and the vestibular cuticle (CLJ). Scale bar = 10 pm. GA, genital aperture; HC, haemocoel.

becomes reduced

4. **Tubular accessory gland**

A pair of tubular accessory glands opens into the vagina at the junction of the vestibular and cervical parts (Fig. 1). In the unfed stage, the small, club-shaped glands (length ~ 100 μm) consist of simple cuboidal epithelial characterized by a heterochromatin-rich nucleus, and externally are surrounded by muscle cells (Fig. 10 inset). The glands increase in size during feeding, then adopt a large paddle-shape (length ~ 800 μm, diam. ~ 200 μm) in the ovipositing stage (Fig. 10). The tall columnar secretory cells have a large round nucleus and in the basal region the cytoplasm is rich in eosinophilic secretory granules (Fig. 11). Coincidentally, there is a development of external muscle layers.

5. **Connecting tube**

The connecting tube opens ventrally at the junction between the vestibular vagina and the cervical vagina, and so links the vagina to the common oviduct (Fig. lb). In the unfed stage, the folded cuticle lined with an epithelial layer is surrounded by thick muscle layers similar to those of the cervical vagina (Fig. 12). During feeding, the epithelial cells become irregularly shaped and the cuticle unfolds as the cells enlarge (Fig. 13). Well-developed muscle layers surrounding the connecting tube overlap each other. No further significant structural changes are observed during oviposition.

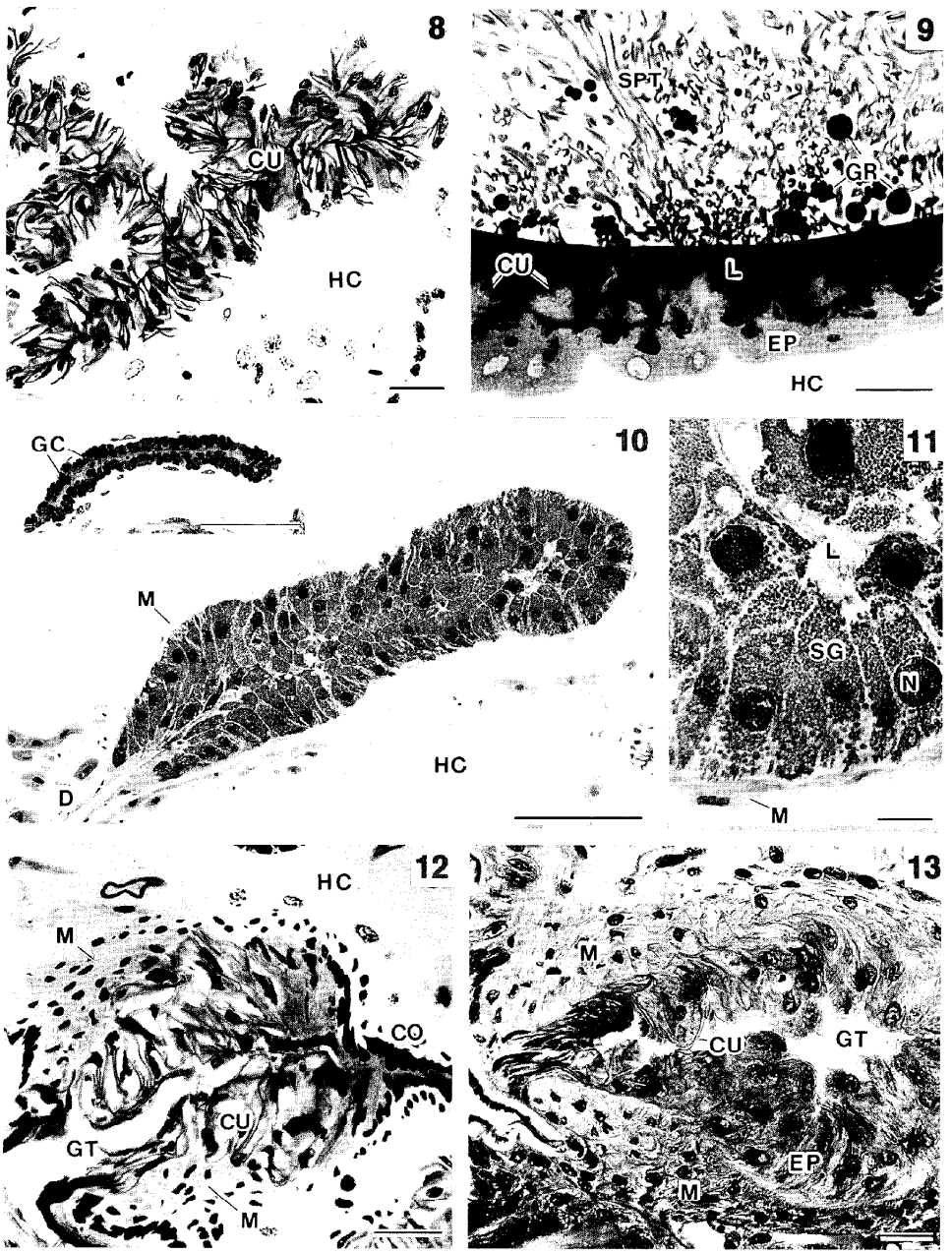
6. **Oviduct**

The regularly arranged oviducal epithelium consists of simple cuboidal epithelial cells surrounded by an undeveloped muscle layer (Fig. 14). During feeding, the cells become columnar and their cytoplasm now contains numerous vesicles and the nucleus is rounded (Fig. 15). During egg passage, the oviducal epithelium is stretched and flattened (Fig. 16).

7. ovary

In the unfed stage, the ovary consists of ovarian epithelial cells with a small nucleus (diam. ~ 5 μm), oocytes with a larger, round nucleus (diam. 15-16 μm) containing a distinct nucleolus, and external connecting tissue (Fig. 17). During feeding, the oocytes protrude into the haemocoel, and are connected to the ovarian surface by funicle cells (Fig. 18). There is no synchrony of oocyte development. The maximum diameter of the oocyte on the 5th day after attachment is about 50 μm and the egg shell has not yet appeared. Just before ovulation in the ovipositing stage, the oocyte has a complete egg shell and yolk (Fig. 19). Numerous spermatozoa ascend into the ovarian lumen.

Figs. 5-7. Light micrographs of sagittal section of the cervical vagina. Fig. 5. The unfed female, showing the folded cuticle (CU) lined with a layer of epithelial cells and the muscle layer (M). Scale bar=10 μm. Fig. 6. The feeding female, just after copulation showing the lumen (genital tract, GT) filled with the substance derived from the endospermatophore inserted by the male. Note the developed muscles (M), epithelial cells (EP) and the expanded cuticle (CU) Scale bar = 10 μm. Fig. 7. The ovipositing female, showing no substance in the genital tract (GT). Scale bar = 10 pm. HC, haemocoel. Arrows, nucleus of epithelial cell.



Figs. 8 and 9. Light micrographs of the receptaculum seminis. Fig. 8. The unfed female, showing the closely folded thin cuticle (CU) lined with an epithelial layer. Scale bar=10 pm. Fig. 9. The distended receptaculum seminis just after copulation showing the endospermatophore containing the spermatids (SPT) and granular structures (GR) inserted into the lumen (L). The cuticle (CU) lined with the developed epithelium (EP) expands with insertion of the endospermatophore. Scale bar=10 pm. HC, haemocoel. Arrows, nucleus of epithelial cell.

DISCUSSION

The structure of the female genital system in *Haemaphysalis longicornis* is basically similar to that of the other families belonging to the Metastriata (Balashov, 1972; Sonenshine et al., 1985; El Shoura, 1989). The endospermatophore inserted from the genital aperture at the time of copulation is carried into the receptaculum seminis through the vagina. Spermatozoa ascend en masse through the cervical vagina, connecting tube and paired oviducts to the ovary where they fertilize the oocytes. Fertilized eggs pass down through the oviducts and connecting tube, and are deposited by eversion of the vestibular vagina.

1. Vestibular vagina

The remarkable development of the lobular accessory glands during oviposition indicates that the secretions from the glands are probably involved in coating the surface of eggs passing through the vestibular vagina. The lipid-rich secretions function as a waterproofing agent (Lees and Beament, 1948). In addition to *Haemaphysalis, Ixodes* (Lees and Beament, 1948 for *I. ricinus*), *Dermacentor* (Sonenshine et *al.,* 1985 for *D. variabilis), Rhipicephalus* (Till, 1961 for *R. appendiculatus)* and *Hyalomma* (Balashov, 1972 for *H. asiaticurn)* all possess these glands, but *Hyalomma dromedarii* lacks them (El Shoura, 1989). The fact that argasid ticks lack the lobular accessory glands (Diehl *et al.,* 1982) may be due to their nidicolous behaviour, unlike ixodid ticks.

2. Cervical vagina

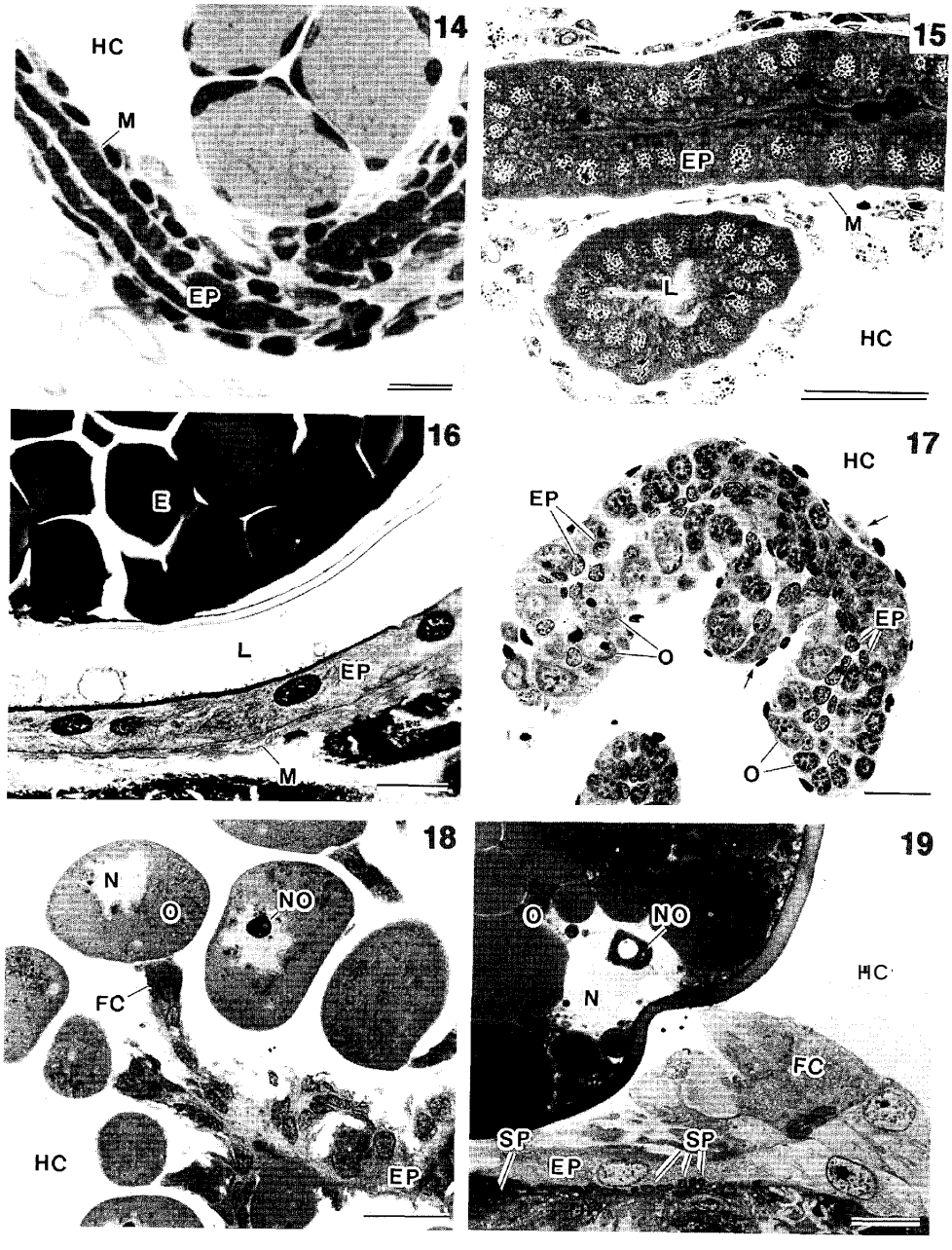
The cervical vagina only connects the receptaculum seminis to the vestibular vagina, the inserted endospermatophore and ascending spermatozoa pass through it, but no eggs pass through the cervical vagina during oviposition. Developed muscle layers which externally surround the cervical vagina probably facilitate the passage of the endospermatophore into the receptaculum seminis, and of spermatozoa to the connecting tube.

3. Receptaculum seminis

The receptaculum seminis is a structure peculiar to the Metastriata in the family Ixodidae (Diehl et *al.,* 1982). The Prostriata, consisting of only 1 genus, *Ixodes,* and the Argasidae do not possess such a separated sac. The role of the receptaculum seminis is filled by the common oviduct in the Prostriata and by the "uterus" in the Argasidae (Diehl

Figs. 10 and 11. Light micrographs of sagittal section of the tubular accessory gland. Fig. 10. The ovipositing female showing the large club-shaped gland surrounded by the muscle layer (M) and narrow duct (D). Scale bar=100 pm. Inset: The unfed female showing the undeveloped glandular cells (GC). Scale bar=50 pm. Fig. 11. Magnified view of the secretory cells of the gland in the ovipositing female. Note the numerous eosinophilic secretory granules (SG). Scale bar=10 µm. HC, haemocoel; L, lumen; N, nucleus.

Figs. 12 and 13. Light micrographs of sagittal section of the connecting tube. Fig. 12. The unfed female showing, the folded cuticle (CU) surrounded by muscle layers (M). Scale bar=10 µm. Fig. 13. The feeding female, showing the expanded cuticle (CU) and irregularly arranged epithelial cells (EP). Scale bar = 10 pm. CO, common oviduct; GT, genital tract; HC, haemocoel.



Figs. 14-16. Light micrographs of the oviduct. Fig 14. The unfed female, showing an oviducal epithelial layer (EP) surrounded by a layer of muscle cells (M) undifferentiated Scale bar=10 pm. Fig. 15. Sagittal (above) and cross (below) section of the oviduct in the feeding female. Oviducal epithelial cells (EP) become columnar and contain numerous vesicles. Scale bar=50 µm. Fig. 16. The ovipositing female, showing an egg (E) just passing through the oviducal lumen (L). Scale bar = 10 pm. HC, haemocoel.

et al., 1982).

The epithelial cells of the receptaculum seminis develop during feeding and may reach a peak of development just before copulation (4-5 days after host attachment). The highly stained secretion-like substances within the cytoplasm of epithelial cells and the lumen of the receptaculum seminis observed just after copulation appear to be involved in destruction of the inserted endospermatophore (Khalil, 1970), after which sperm ascent commences. Spermiogenesis of ixodid ticks starts in the male body and it continues also in the female tract after copulation (Oliver, 1982). In Ambl *yomma hebraeum*, spermiogenesis is completed during storage in the receptaculum seminis when mature spermatozoa first appear (El Said et al., 1981).

4. **Tubular accessory gland**

A pair of tubular accessory glands is a common feature in the Ixodoidea: Ixodidae (Lees and Beament, 1948; Till, 1961; Roshdy, 1969; Balashov, 1972), Argasidae (Lees and Beament, 1948; Roshdy, 1961; El Shoura, 1988) and Nuttalliellidae (El Shoura et *al.*, 1984). Histochemical studies of the glands in *Hyalomma asiaticurn* (Balashov, 1972) and *Haemaphysalis spinigera* (Chinery, 1965) indicate that secretions from the glands contain basic protein. It is considered that these proteinaceous secretions act as a waterproofing agent for the egg surface and/or as a lubricant that facilitates egg passage (Robinson and Davidson, 1914; Douglas, 1943), as do those of the lobular accessory glands.

5. Connecting tube

Well-developed muscle layers which externally surround the connecting tube probably facilitate the active transfer of a mass of spermatozoa into the oviduct and of eggs into the vestibular vagina.

6. **Oviduct**

Numerous vesicles observed in the oviducal epithelial cells just after copulation presumably contain secretory products which, when released, may exert some influence upon the passing spermatozoa and eggs. In *D. andersoni*, the oviducal epithelia have well-developed microvilli and cell processes of the apical surface. It has been suggested that both may help to protect them against the intracellular invasion of spermatozoa (Brinton *et al.*, 1974). These structures are present also in the oviduct of *Haemaphysalis longicornis* (Kakuda *et al.*, unpublished). In *Hyalomma dromedarii* (El Shoura, 1989) and *D. andersoni* (Brinton et *al.*, 1974), oviducal epithelial cells display granular material which we presume is involved in sclerotization of the egg's shell during its passage. Muscle layers surrounding the oviduct effect peristaltic contractions during the passage of both spermatozoa and eggs.

Figs. 17-19. Light micrographs of the ovary. Fig. 17. The unfed female. The ovary consists of oocytes (0) with large round nucleus and ovarian epithelial cells (EP) with small nucleus, surrounded by a layer of connecting tissue (arrows). Scale bar = 20 µm. Fig. 18. The feeding female. Oocytes (0) protruding into the haemocoel (HC) connect to the ovarian wall by the funicle cells (FC). Scale bar = 20 pm. Fig. 19. The ovipositing female. A fully developed oocyte (0) connecting to the ovarian epithelium (EP) by the funicle cells (FC). Numerous spermatozoa (SP) ascend into the ovarian lumen. Scale bar = 10 pm. N, nucleus; NO, nucleolus.

7. ovary

Just after copulation and so corresponding to the period of great cytoplasmic growth (Balashov, 1972), the oocytes remain associated with the ovarian surface by the funicle cells. Whether the funicle cells actually originate from ovarian epithelial cells and whether they have already differentiated in the unfed stage or not, are questions that can only be answered by ultrastructural studies.

The site of fertilization in ticks is still a point of contention. Balashov (1972) concluded that oocytes are fertilized in the anterior part of oviducts. However, in *D. andersoni* (Brinton and Oliver, 1971) and *Haemaphysalis* longicornis (present study), spermatozoa exist in the ovarian lumen at the beginning of oviposition; furthermore, some are wedged into an indentation of the funicle plasma membrane in *H. longicornis* (Yano et al., 1989). These facts imply that fertilization occurs within the tick ovary. However, it remains unclear whether the actual site of fertilization is the ovarian lumen or the intercellular space of funicle cells, or whether fertilization occurs as a result of the penetration of the spermatozoon through the egg shell just before ovulation.

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