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Ultrastructural Study During Feeding and Mating on the Genital System in Female Tick Hyalomma (Hyalomma) anatolicum anatolicum (Ixodoidea: Ixodidae)

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Abstract. The fine structure of genital system in the female *Hyalomma (Hyalomma) anatolicum anatolicum* is described during feeding and mating. Feeding initiates oocytes growth through the previtellogenic. Oogenesis is induced by feeding . Growing oocytes secreate dense granules into the cytoplasm and form surface microvilli. The cytoplasm has associated dense vesicles, Golgi bodies, rough endoplasmic reticulum and multivesicular bodies.

The vagina, consisting of vestibular (VV) and cervical (CV) regions, is formed of an epithelium lined internally with a folded cuticular layer and surrounded externally by muscle layers. These facilitate the passage of endospermatophres containing sperms into the receptaculum seminis (RS), and ova to the exterior. A pair of tubular accessory glands (AG) opening at the junction of VV and CV consist of an epithelial layer of undifferentiated cells. As feeding progresses, these cells synthesise their granular secretion that functions to lubricate the egg surface during its passage through VV. The receptaculum seminis consists of cuboidal cells lined with a cuticular layer. These cells appear rich with glycogen and lipid vacuoles, may act as source of energy required for various cell activities including granule synthesis and exocytosis. The granules discharge their contents into intercellular spaces distributed throughout the wall of the receptaculum seminis, and communicate with the main lumen *via* narrow channels. No secretory activity has been observed during and after feeding in the connecting tube. At and during final stage of feeding, the cell cytoplasm contains large, phagosomal vacuoles penetrated by sperms, in addition to micropinocytotic vesicles which serve to break down the seminal fluid and other material. The oviducal secretion may function as a tanning agent to harden the egg shell and also probably act as a lubricant at the state of egg passage. In addition, the basal membrane is infolded giving characteristic features of epithelia involved in ion water transport.

Keywords: Ixodid tick Genital tract, electron microscope.

Introduction

Ticks are obligate ectoparasites which infest every major vertebrate animal group, including man, and feed on their blood. They are reservoirs and vectors of numerous pathogenic viruses, rickettsia, bacteria, protozoa and filaria[1].

Ixodid ticks are highly specialised bloodsucking arthropods. The tick is known to be one of the important reservoir and vectors of microorganisms causing diseases to livestock and wild life [2]. The ticks have the ability to transmit certain pathogens to their progeny *via* transstadial and transovarial infection[3].

Hyalomma (H) anatolicum anatolicum is common in Saudi Arabia wherever the camels occur. It is very important ectoparasite which infests the majority of camels and can also feed on humans.

The anatomy and histology of the female reproductive organs have been investigated in several ixodids[2], [4-7] and argasids[8,9].

The ultrastructure of the developing oocytes has been described in the ixodid ticks *Rhipicephalus bursa*[10], *Dermecentor andersoni*[11], *Hyalomma asiticum*[12];[13], *Amblyomma hebraeum*[10];[12] and *Hyalomma anatolicum*[7]. The general pattern is the same in each of these species. Juliana *et al*[15]. showed that the ovary of the tick *amblyomma triste* is classified as panoistic, which is characterized by the presence of oogonia without nurse and follicular cells.

Brinton *et al*[16]. have described the fine structure of the oviducts in relation to spermiogenesis in the oxidid ticks Dermacentor andersoni, and Raikhel[17] has studied briefly the effect of blood meal and mating on the reproductive system in *Hyalomma asiaticum*. El-Shoura[18] described the structure of female genital organs, including the egg waxing apparatus, the Gene's organs, in unfed *O. erraticus*. The fine structure and mechanism of the Gene's organs in feeding and ovipositing *H. dromedrii* was also revealed by El-Shoura[19]. El-Shoura *et al.*[20] described that certain genital organs in the nuttalliellid tick, Nuttalliella namaqua, combine features of argaside and ixodid ticks.

The female genital organs of the tetrablemmid Indicoblemma lannaianum are astonishingly complex[21]. The copulatory orifice lies anterior to the opening of the uterus externus and leads into a narrow

insertion duct that ends in a genital cavity. The genital cavity continues laterally in paired tube-like copulatory ducts, which lead into paired, large, sac-like receptacula[21].

The Present Study

The present study describes the ultrastructure of the female genital organs in the tick *Hyalomma (Hyalomma) anatolicum anatolicum* during feeding and mating, and up to oviposition to establish a basis for additional future research incorporating biochemical and cytochemical approaches to increase our knowledge of chemotactic processes influencing sperm.

Materials and Methods

The specimens of *Hyalomma (H) anatolicum anatolicum* used in this study were collected mainly from abattoirs and camel markets at Jeddah and Makkah in Saudi Arabia, particularly the Muna and Arafat areas and maintained at 28 ºC and 75% relative humidity. Domesticated rabbits were used as laboratory hosts. Unfed (7-day-old), feeding (3, 5 and 7 days after attachment), Fully engorged (3, 5 and 7 days after detachment), and ovipositing females were dissected in insect physiological saline. The genital system was released from surrounding connective tissues and separated into organs, each of which was fixed individually in 3% glutaraldehyde in Na-cacodylate buffer (pH 7.2), postfixed in an alcohol series and embedded in Spurr resin [22]. Semithin sections were stained with 1% toluidine blue. For transmission electron microscopy (TEM) ultrathin sections were double-stained with uranyl acetate and lead citrate and examined under a Philips 400 TEM. Images were recorded on Ilford E.M cut film and printed on Ilfospeed multigrade paper. (More details in Bughdadi[23,24].

Results

Ovary

The blood meal and mating initiates the resumption of oogenesis. The ovary starts to increase in both length and diameter. It thus enters the great cytoplasmic growth phase (stage II). This increase in size is due to

the differentiation of most of the oogonia to primary oocytes; to the enlargement of already existing primary oocytes and to the protrusion of the oocytes from the ovary into the body cavity. As the oocytes increase in size, these connecting (funicle) cells become columnar and, with the oocytes, extend into the body cavity (Fig. 1), carrying their connective tissue sheath, or tunica propria, before them (Fig, 2).

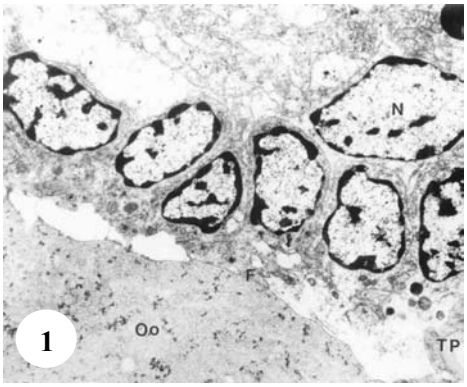

Fig. 1. Transverse section showing the funicle cells (F) which connect the oocyte (Oo) with the ovarian wall. Note tunica propria (TP). X 15,000.

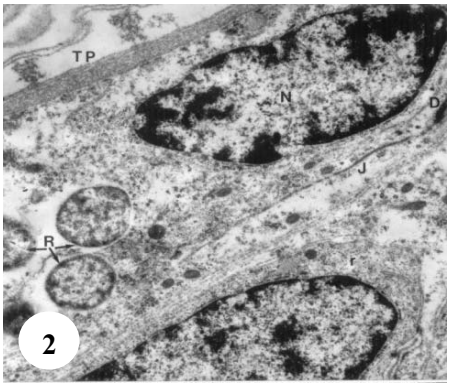

Fig. 2. Transverse section showing the funicle cells containing rickettsia-like microogranisms (R), desmosome (D), junction complex (J), free ribosomes (r), and connective tissue sheath of tunica propria (TP). X 7,000.

The oocytes cell cytoplasm is rich in free ribosomes, membrane-bounded electron-lucent vacuoles, numerous small mitochondria, lipid droplets, and single rickettsia – like microorganisms (Fig. 3, 4). Micropinocytotic coated pits appear in the oocytes cell membrane between the bases of the microvilli (Fig. 4). The adjacent finical cells are joined by long, tortuous desmosomes, junctional complexes and numerous mature spermatozoa are observed in the ovarian lumen (Fig. 5,6). The oocytes show further development changes. Further changes in the nucleus; the beginning of cytoplasmic changes, including those associated with primary yolk granules formation (vitellogenesis) and early stages in the formation of egg shell (vitelline envelope).

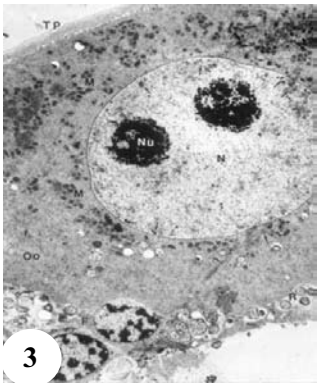

Fig. 3. Part of oocyte (Oo), showing nucleus (N), two nucleoli (Nu), mitochondria (M), ribosomes (r), fine granular euchromatin stars) in the nucleoplasm, ribosomal - like granules close to the nuclear evelope and in the cytoplasm (arrows). Note the rickettsia-like microorganisms (R) and part of funicle cells (F). X 6,000.

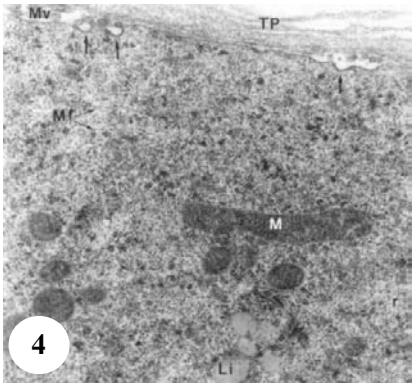

Fig. 4. Cytoplasm showing mitochondria (M), lipid droplets (Li), free ribosomes (r), and microfilamentous material (Mf). Note the microvilli (Mv) on the surface of the oocyte underlying the tunica propria (TP) and the coated pits arrows). X 16,500.

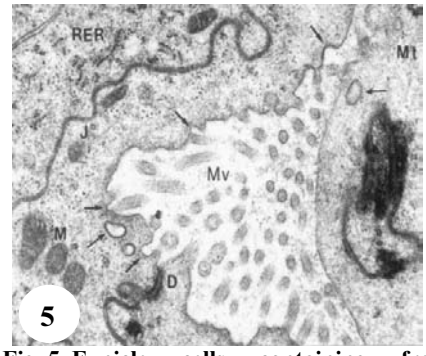

Fig. 5. Funicle cells containing free ribosomes (r), mitochondria (M), microtubles (Mt), rough ER (RER). The apical microvilli (Mv) project into the ovarian lumen and are coated with glycocalyx. Note also coated vesicles and pits (arrows), junction (J), desmosome (D). X 37,500.

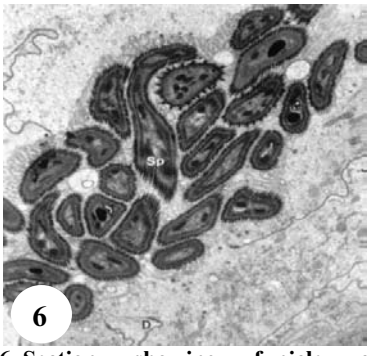

Fig. 6. Section showing funicle cells surrounding numerous mature sperms (Sp) in the ovarian lumen. Note the junctions (J) and desmosomes (D). X 12,500.

In the ooplasm, large patches of condensed and loose electron-dense ribosomal aggregates are in close association with the nuclear envelope (Fig. 3, 7). Single rickettsia-like microorganisms are also present in the cytoplasm (Fig. 7).

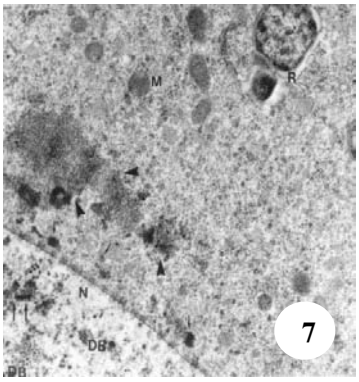

Fig. 7. Part of cytoplasm and nucleus (N) with ribosomal-like granules in the nucleoplasm and in the cytoplasm near the nuclear envelope (arrows), mitochondria (M), rickettsia-like microorganisms (R), granular-ribosomal aggregates arrowheads) and dense bodies (DB). X 7,400.

Vagina

During feeding, the entire genital system enlarges gradually in size during feeding and mating (attachment), and rapidly after the tick detachment to reach its maximum growth during oviposition. However, no remarkable structural changes were observed in both VV and CV during feeding (Fig.8). During oviposition, VV cuticular folds become considerably unfolded due to the enormous stretching of the entire cuticular lining, which in some areas appear lamellate. Degenerated epithelial cells of CV, as those of VV, are electron-dense with pycnotic nuclei (Fig. 9).

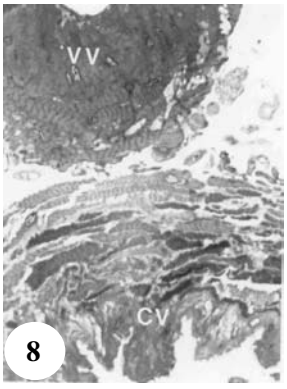

Fig. 8. Semithin sections of vestibular vagina (VV) and cervical vagina (CV)during feeding. X 1,200.

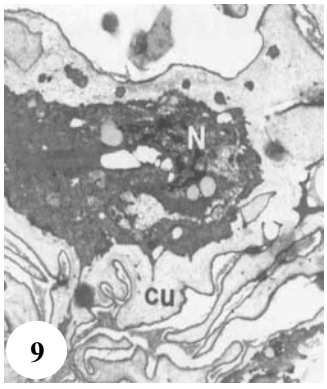

Fig. 9. Degenerated epithelial cells of cervical vagina (CV) showing pycnotic nucleus (N). cu, cuticular lining. X 4000.

Tubular Accessory Glands

As feeding progresses, the glandular epithelial cells become pyramidal with many ultrastructural features indicating secretory activity. The cytoplasm becomes packed with well-developed rough endoplasmic reticulum (RER) which is apparently responsible for the formation of electron-dense granules distribution throughout the cytoplasm (Fig. 10). The cell apices containing secretory granules and mitochondria project numerous, elongated, contiguous microvilli enclosing peripheral, longitudinally oriented fibrillar material within their cortex (Fig. 11). Some pinocytoticpits are detected in the apical cell membrane (Fig. 11). The cell basal region is infolded (Fig. 12). The nuclei are greatly enlarged and located basally; their chromatin consistency becomes loose and the dense clump, seen in unfed ticks, is remarkably decreased; the nucleolus granular components are abundant (Fig. 12).

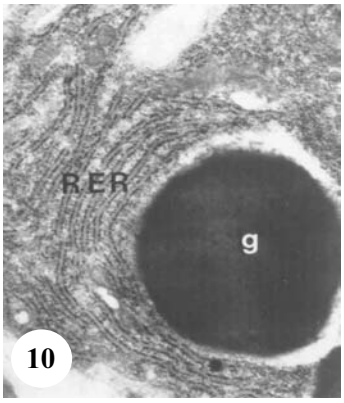

Fig. 10. Basal region of accessory gland (AG) during feeding showing a secretory granule (g) surrounded by well-developed rough endoplasmic reticulum (RER). X 25,500.

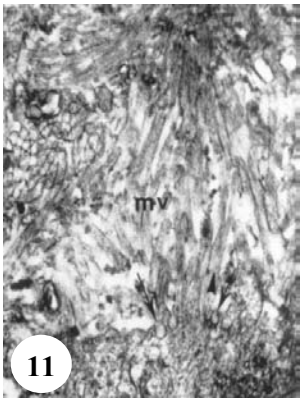

Fig. 11. As in figure 10 but showing the apical region with elongated microvilli (mv) enclosing peripheral fibrillar material (arrowheads). Note pynocytotic pits (arrows). X 40,000.

During oviposition, the pyramidal cell cytoplasm contains secretory granules of variable dense, size and structure (Fig. 13), and may represent different granular growth phases. An electron-dense material identical to that of dense granules is found in the gland lumen and closely associated to the cell apices (Fig. 14). Abundant microtubules occupy most of the cell apical regions lying perpendicular and closely attached to the heavily microvillate apical plasma membrane (Fig. 14).

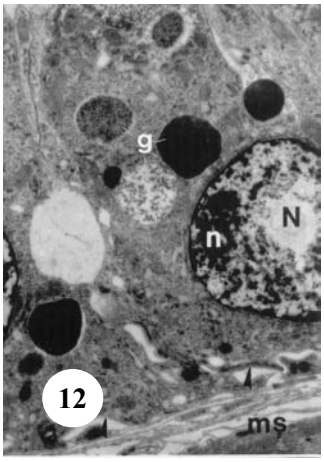

Fig. 12. Basal region of AC during feeding showing enlarged nuclei (N) with loose chromatin consistency and decreased dense heterochromatin seen in unfed ticks (see [7]). Note basal membrane infoldings (arrowheads), scattered granules (g) and muscle layer (ms). n, nucleolus. X 11,000.

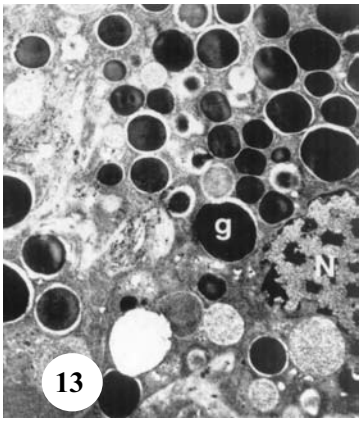

Fig. 13. AC during oviposition showing the cell cytoplasm of pyramidal containing secretory granules (g) of changeable dense, structure and size. N, nucleus. X 3,500.

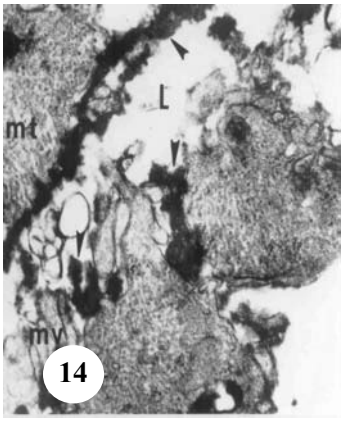

Fig. 14. AC during oviposition showing abundant microtubules (mt) in the cytoplasm of the apical region. Note dense material (arrowheads) in the lumen (L)of the gland and it seems that released through microvilli (mv). X 40,000.

The Receptaculum Seminis

Within few days of feeding and mating, and after the endospermatophores have been injected into receptaculum seminis lumen, the epithelial wall becomes greatly distended while the cuticular lining appears interrupted (Fig. 15). In advanced cells, Golgi apparatus

giving rise to vesicles and closely associated with rough endoplasmic reticulum, in addition to free ribosomes and elongate mitochondria, are observed in the cell cytoplasm (Fig. 16). The columnar cell is filled with glycogen particles (Fig. 15&17) characterized by their identical rosette shape, which in some areas, surround lipid-like vacuoles (Fig. 17). In middle regions of the receptaculum seminis wall, many intercellular spaces are formed by surrounding cells, the apices of which form well-developed microvilli (Figs. 18). These spaces are filled with a moderately dense secretion (Fig. 18&19), and join the receptaculum seminis main lumen (Fig. 15). The irregularly outline nuclei (Fig. 19) occupy great parts of the cells. The dense granules discharge their content into the intercellular space, after fusion of their limiting membranes with microvillate apical membrane (Fig. 18&20), and then into receptaculum seminis main lumen (Fig. 15). Coated pits and vesicles containing fine granular material, in addition to many elongate mitochondria, free ribosomes, and lysosome-like bodies are detected in the cell apical regions (Fig. 21). Lateral cell membranes are joined by long, septate junctions (Fig. 18).

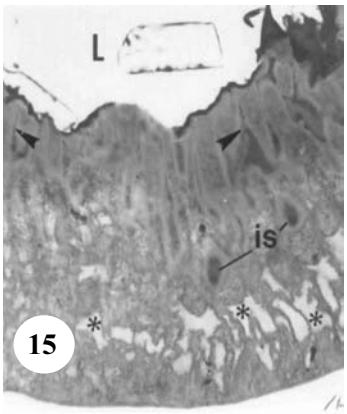

Fig. 15. Semithin section through receptaculum seminis (RS)during feeding showing narrow channels (arrowheads) communicating intercellular spaces (is) and main lumen (L). Note variable size of vacuoles glycogen which was filled it, but it seems washed out during tissue processing. Note the point of interrupted cuticle (arrows). X 1,200.

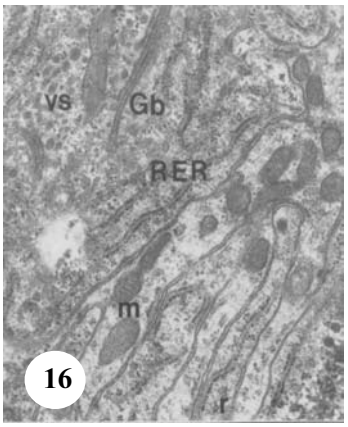

Fig. 16. Advanced RS cells during feeding showing Golgi bodies (Gb) giving rise to small vesicles (vs) and free ribosomes (r). X 25,000.

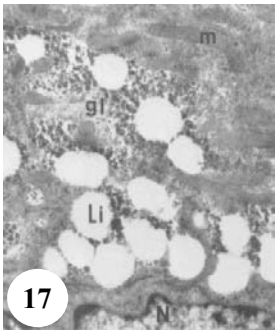

Fig. 17. Ultrathin section in RS showing lipid-like vacuoles (Li) surrounded by glycogen particles (gl). Note m, mitochondria; N, nucleus. X 15,000.

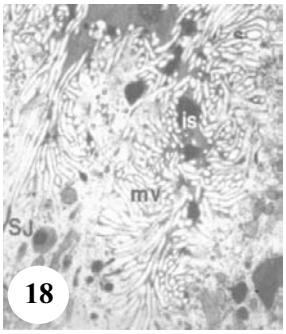

Fig. 18. Apices of adjacent RS cells projecting well-developed microvilli (mv) and joined by septate junction (SJ). Note intercellular space(is). X 11,000.

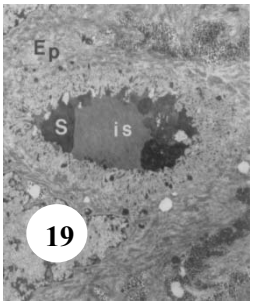

Fig. 19. Middle region of receptaculum seminis (RS) wall showing an intercellular space (is) formed by surrounding cells (Ep). Note nucleus (N), secretory product (S). X 20,000.

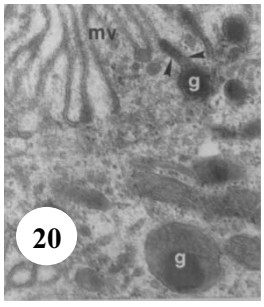

Fig. 20. Ultrathin section showing higher magnification of middle region in figure 18. Note exocytosis and granules (g) content is discharged into the intercellular space after the fusion of their limiting membranes (arrowheads) with microvilli (mv0. X 35,000.

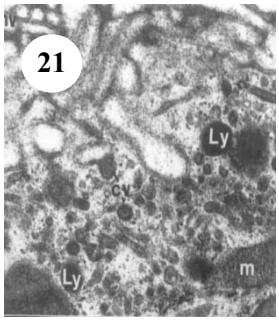

Fig. 21. Similar to figure 20 but this section showing pinocytotic vesicles (cv). Ly, lysosome-like structures; m mitochondria; mv, microvilli; r, free ribosomes. X 35,000.

Oviduct

During the attachment and feeding, epithelial cells become numerous in the entire oviduct (Fig. 22). As attachment and feeding progresses, the oviduct cells differentiate into pyramidal to columnar (Fig. 23), and the most characteristic feature of this epithelium is the penetration of their cell vacuoles by sperms (Fig. 24). The sperms are present in close association with large, membrane-limiting vacuolar structures filled with moderately dense, fine granular material (Fig. 24). The main oviduct lumen, in some regions, is divided into small, intercellular spaces by the microvillate apical cell protrusions (Fig. 25). Pinocytotic pits and vesicles (Fig. 26), rough endoplasmic reticulum (RER), elongate mitochondria, and lysosome-like structures are present in the apical regions (Fig. 27). Lateral cell membranes are joined by long desmosomes and septate junction (Figs. 25&26), while the basal membranes form deep infoldings (Fig. 28) enclosing mitochondria, dense bodies, RER, and microtubules (Fig. 28). Many dense granules are detected in the cytoplasm, and probably discharge their content into the main duct lumen through the long, brush-border, apical microvilli (Fig. 29). The cell of the external epithelium differentiates into a layer of muscle fibres (Fig. 23), which cause peristaltic contractions of the entire oviduct during the passage of both sperms and eggs.

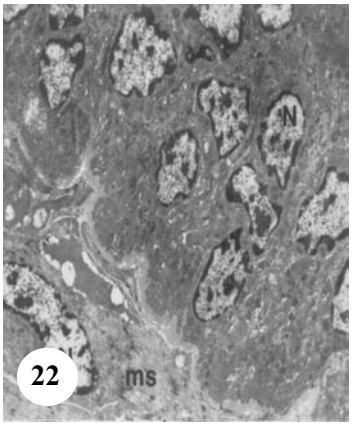

Fig. 22. Ultrathin section of oviduct (OV) during feeding showing the cytoplasm contain numerous cells. Note muscle layer (ms) and the nucleus (N). X 10,000.

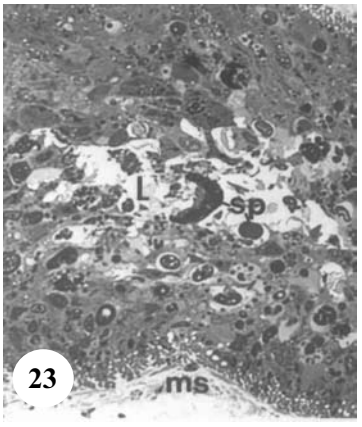

Fig. 23. Semithin section through the common oviduct (COV) showing cells invaded by sperm (sp). Note the lumen of the oviduct (L) and muscle layer (ms). X 1,200.

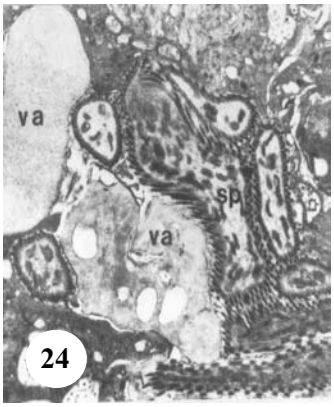

Fig. 24. Ultrathinsection through common oviduct showing vacuolar structures (va) in close association with sperms (sp). X 4,500.

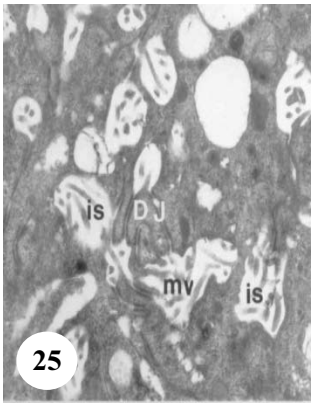

Fig. 25. Section of the main lumen of the oviduct subdivided into smaller intercellular spaces (is) by microvillate (mv) cell apical protrusions. Note septate junction of desmosome (DJ). X 15,000.

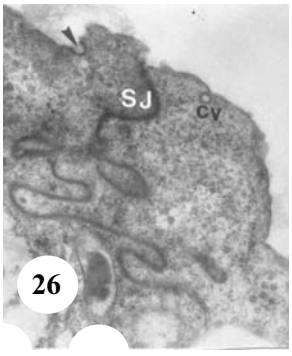

Fig. 26. Oviduct cell apices containing pinocytotic pits (arrowheads) and vesicles (cv) and joined by long septate junction (SJ). Note vacuoles (va). X 25,000).

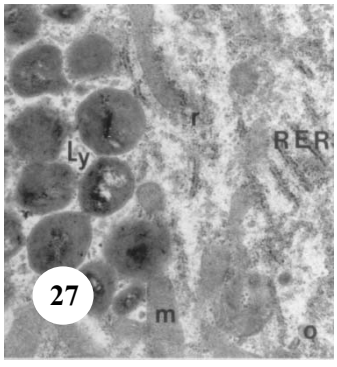

Fig. 27. Oviduct cell apices showing rough endoplasmic reticulum (RER), free ribosomes (r), mitochondri (m) and lysosome-like structure (Ly). X 20,000.

The Connecting Tube

The epithelial cells of connecting tube are elongated and project a few, short microvilli beneath the thin cuticular lining. No sign of secretory activity has appeared in their cytoplasm during or after feeding. However, numerous rickettsia-like microorganisms are present in the cytoplasm (Fig. 30). The nuclei are present and occupy different levels in the connecting tube wall (Fig. 30).

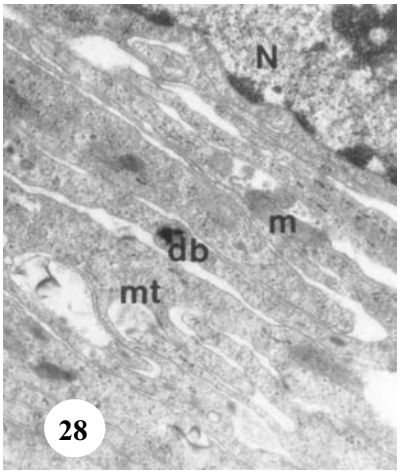

Fig. 28. Cell basal region of oviduct showing basal membrane infoldings enclosing mitochondria (m), dense bodies (db), RER and microtubules (mt). X 22, 500.

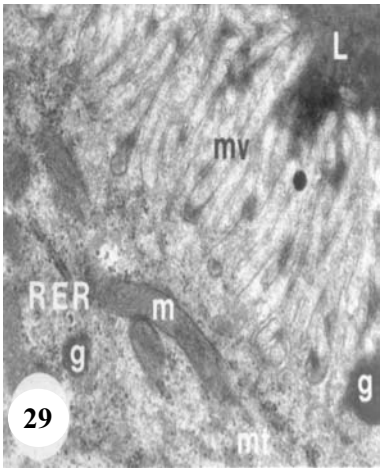

Fig. 29. Cell apical region of the oviduct during oviposition showing dense granules (g) and brush-border microvilli (mv). Note the lumen (L); mitochondria (m); microtubules (mt) and rough endoplasmic reticulum (RER). X 25,000.

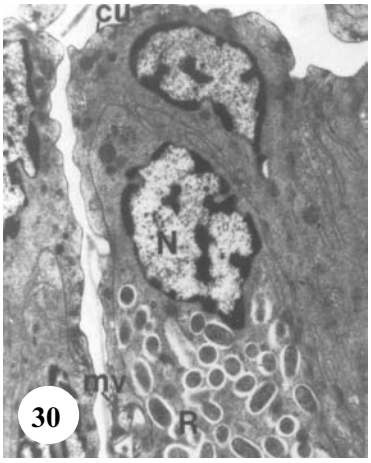

Fig. 30. Ultrathin section of connecting tube at final stages of feeding showing enlarged epithelial cells projecting a few microviili (mv) beneath the thin cuticular lining (cu). Rickettsia-like microorganisms (R) are present in close association to nucleus (n), note the lumen (L). X 11,000.

Disussion

The ovary

The structure of the ovary appears similar to that of other ixodid and argasid ticks at the electron microscope level. In the present study, the ovarian wall of the unfed female consists of interstitial cells, dark oogonia and young oocytes. These three components have been described also in *H. Asiaticum*[13], *H. Dromedrii* [23] and *H .anatolicm*[7].

Oogenesis has been described in the ixodid tick, *Rhipicephalus bursa*[25], *Dermacentor andersoni*[11], *Hyalomma asiaticum*[14] and *Amblyomma hebraeum* [10&12] and in less detail in the argasid *Ornithodoros moubata*[6, 10]. The general pattern is the same in each of these species. Balashov[1,13] has identified five stages of oocyte development. Stage I is the oogonial development in immature stages leading to the formation of young oocytes in newly moulted, unfed females; this is a stage of "small cytoplasmic growth" [13]. Stage II is the period of "great cytoplasmic growth" of the oocyte initiated by the adult blood meal. This stage is the beginning of active enlargement of the nucleus and nucleolus, and formation of microvilli on the surface of the oocytes. The cytoplasmic organelles undergo intense development in this growth phase; the nucleus enlarges and the nuclear envelope is well-defined. The nucleoplasm contains fine granular or fibrillar euchromatin material and the nucleolus despatches dense ribosomal like granules into the nucleoplasm. The oocyte cytoplasm is rich in free ribosomes; some mitochondria and numerous rickettsia-like microorganisms. In this stage the oocyte cell membrane forms microvillar projections over the whole surface. Stage III begins with the primary yolk granule formation. In stage IV, these granules are much enlarged and increased in number. Their full development marks the end of stage IV, when they fill the oocyte cytoplasm. Stage V represents the ovulated mature egg in the ovarian lumen, oviduct and uterus. Stage II is also referred to as previtellogenic development, and stages III and IV are the vitellogenic development of oocytes[14].

Knowledge of oocyte development in ticks is essential to the understanding of the biomedical and epidemiological importance of vector potential, and the capability for transovarial transmission of several human and animal diseases agents to their progeny.

During feeding and attachment, the ovary increases in size, and becomes folded along its length. This increase in size has been described in the argasid ticks *Argas persicus*[12] and *Argas arboreus* [26], *Argas arboreus* [8], and the fold has been described in the ixodid ticks *R. Appendiculatus* [27], *D. Andersoni* [5 and 11], and *H. Asiaticum* [1,13]. In *H. dromedarii* and the other species studied, this increase in size is associated with the onset of oogenesis. In *D. Andersoni* [5,11], the oocytes in the longitudinal fold are considerably smaller than those outside it. In *H. dromedarii*, oogenesis is asynchronous, so that different stages of development can be observed in the same ovary.

According to Brinton and Oliver[5,11], this asynchronous development is probably due to the feeding processes, thus the ovary increases greatly in size and becomes folded. The oocytes outside the longitudinal fold are more active, than those in the longitudinal fold. However, Brinton and Oliver [5,11] reported that asynchronous development is due to inhibitory effects of oocyte crowding in the longitudinal folds. This asynchronous development occurs also in *R. Appendiculatus*[27], *H. anatolicum*[4]; *D. Andersoni*[5,11] and *H. asiaticum*[1,13].

The formation of the funicle cells in the ovarian epithelium of *H. anatolicumi* marks the beginning of the cytoplasmic growth phase (Stage II) of Balashov[1,13] and [14]. They are a feature also in other ixodids and argasids ticks [6,10]. These connecting (funicle) cells proliferate, become columnar and, with the oocytes, extend into the body cavity carrying their connective tissue before them. Some authors have suggested that funicle cells may be a source of heterosynthetic yolk protein[6]. Others have considered that the funicle serves only to attach the oocyte to the ovarian wall [5,9,11].

Coated vesicles similar to those seen in the funicle cells of *H. anatolicum* during feeding and attachment animals have been described in the funicle of the ixodid *Rhipicephalus bursa* [10] and the argasid tick *Ornithodoros moubata*[6]. Balashov [13] suggested that these vesicles may participate in the passage of high molecular substance. Their presence does suggest that the funicle cells may have more than simply a support function.

The mature sperms seen in the ovarian lumen of *H. anatolicum* presumably passed through the oviduct. Sperms have also been seen in

the ovarian lumen of *Dermacentor andersoni* [16]. These mature sperms probably penetrate the epithelial funicle cells, which are probably a passage way for spermatozoa to gain access to impregnate the oocyte permitting transfer of spermatozoal nuclei into the oocytes.

The period of great cytoplasmic growth following the tick blood meal includes dramatic changes in the oocytes organelles. The oocyte is clearly entering a phase of active protein synthesis. The emission of dense granular material from the nucleolus, and its passage to the cytoplasm becomes apparent in this stage. The histochemical studies on the developing oocytes of some tick species have demonstrated the presence of basophilic granular material, interprated as ribonucleoproteins in the nucleus and perinuclear cytoplasm [12,14]. Diehl *et al.* [14] interprets these granules to be ribosomal precursor gaining access to the cytoplasm through nuclear pores. This interpretation is supported by the observation on *H. anatolicum* Golgi bodies in the form of scattered dictyosomes associated with dense vesicles which fuse to the ribosomal aggregates. In present paper the electron dense stacked cisternae and the vesicles derived from the Golgi complex are probably involved in the production of multivesiculate bodies. Brinton and Oliver[11], suggested morphogenetic mitochondrial changes in *D. andersoni* are involved in the production of membrane-limited multivesiculate bodies. In *H. anatolicum* such mitochondrial changes have not been seen. Moreover, in the present study both the ribosomal aggregates and the multivesculate bodies are associated with the formation of primary yolk granules which consist mainly of proteins. These are similar to that in *Ornithodoros moubata* [9]; *D. andersoni* [12] *H. Asiaticum* [13] and in some ixodids and argasids ticks [14]. According to these authors, the granular ribosomal aggregates, the small vesicles and multivesculate bodies present in *H. anatolicum* oocyte cytoplasm are all involved in the formation of the yolk granules. The involvement of mitochondria, Golgi vesicles, nucleolar emission and ribosomes in the formation of primary yolk bodies has been reported for several insect and arachnid oocytes[13]. In insects, follicle cells are involved in the formation of yolk granules[28] and facilitate the transmission of yolk precursors from the haemolymph. In the ixodid *Haemaphsalis spinigera*[29] found that the yolk bodies in the oocytes were tyrosine-rich basic proteins linked to carbohydrates. However, the histochemical[9] and biochemical[30] investigations have demonstrated that the egg yolk

proteins in several species of ixodids and argasids are haemo-glyco-lipoproteins. Yolk formation in tick oocytes is affected by both intraoocytic and extraoocytic synthesis [6,13,14]. Intraoocytic synthesis is concerned with activities of the cell organelles, as mentioned above. Extraoocytic synthesis is concerned with the formation of the oocyte surface.

In *H. anatolicum* the oocyte surface develops microvilli. A similar appearance of the small microvilli underlying the tunica propria in the early stages of oogenesis has been described in *Dermacentor andersoni* [5,11] and *H. asiaticum* [13] . These surface microvilli in the growing oocytes may facilitate micropinocytosis of nutrient material from the haemolymph, which can penetrate through the basal lamina investing the ovarian epithelial cells. The appearance of micropinocytotic pits and vesicles in the ovarian epithelial cell cytoplasm, as well as the presence of micropinocytotic pits, vesicles, and tubes in the oocyte peripheral cytoplasm of both *H. anatolicum* and other ticks species [13+14], supports an extraoocyte source of yolk material which is internalized into the oocyte. The fusion and condensation of such vesicles and reservoirs could form membrane-limited, finely granular bodies[14] seen in *H. anatolicum* and other ticks. It seems likely that these fuse to the primary yolk bodies, thus forming the large yolk spheres which gradually fill up most of the oocyte cytoplasm. Future studies are needed to increase our knowledge about the full story of the oogenesis and the formation of the egg shell.

Vagina

The structure of the vestibular vagina of *H. anatolicum* is basically similar to that of *H. asiaticum*[17]. In some ixodids the epithelium of the vestibular vagina during feeding detaches from the cuticle and becomes glandular. However, during oviposition, the lipid-rich secretion is discharged, through the cuticle, into the lumen of the gland and onto the surface of the passing egg which becomes partially waterproof [31]. Such a vestibular epithelial gland has not been observed during the feeding and mating in *H. anatolicum*. Furthermore, except for Ixodes species, the cervix in *H. anatolicum* is structurally similar to those observed for other ixodids[1,17,27] , they have a separate receptaculum seminis opening into the cervical vagina. In *Ixodes ricinus*[12] and *H. dromedarii*[32] , a common oviduct acting as a receptaculum seminis opens *via* a short

connecting tube into the dorsal surface of the cervical vagina. As in other tick species, the cervical vagina in *H. anatolicum* is surrounded by several, well-developed muscle layers which probably facilitate the passage of endospermatophores containing sperms into the receptaculuim seminis, and ova to the exterior. Recent research recorded that in some feeding ixodids the epithelium of the vestibular vagina detaches from the cuticle and becomes glandular. Moreover, during oviposition, the lipid-rich secretion is discharged, through the cuticle, into the lumen of the gland and onto the surface of the passing egg which becomes partially waterproofed[15].

Tubular Accessory Glands

The accessory glands in *H. anatolicum* have synthesised their secretory granules only after attachment to the host, during feeding and mating, while in other argasids ticks[18,33], the glands already contain secretory granules before feeding. This may be due to the remarkable behavioural differences between the two species. In *H. asiaticum,* histochemical studies have shown that these glands secrete a colloidal material containing basic proteins[1], which presumably coats the egg surface during its passage through the cuticular vagina. The presence of microtubules in the present study is similar to that reported in *H. asiaticum*[1]. These microtubules are probably involved in a mechanical role responsible for discharging the granular content into the gland lumen.

The Receptaculum Seminis

The receptaculum seminis is present only in ixodid ticks, and develops only during feeding and mating. Although, the fine structure and development of this organ in *H. anatolicum* is similar to that in *H. adromedrii*[23], but is entirely different from that of *H. asiaticum*[17]. The intercellular spaces which collect secretory products from surrounding cells and passing them, through narrow channels, into the main lumen of the organ, where the endospermatophores are present, may function as pooling systems; these probably serve to protect the microvillate cell apices from the direct contact with the endospermatophores and probably to control the flow of the cell secretion into the main lumen through the narrow channels. Enormous microvilli and infoldings basal membrane observed in receptaculum seminis cells may be characteristic features of transporting epithelia[34]. Although final stage of sperm maturation,

spermiogenesis[35], present in the female genital system [26,36], sperm should be released after the digestion of endospermatophore wall, probably by the secretion of the receptaculum seminis. The presence of the rough endoplasmic reticulum and Golgi bodies in the present paper are apparently involved in the formation of the secretion of receptaculum seminis. Moreover, the lysosome-like structures found in the cell apices may also play an important role in dissolving the wall of endospermatophore, and / or function in the break down of material taken up by pinocytosis.

The material of endospermatophric consisting of mucoproteins (Tatchell[37]), and other material taken up pinocytosis could undergo interacellular enzymatic digestion following rupture of these lysosomal-like shape. Exocytosis and endocytosis require an energy consumption which is probably supplied by the great amount of glycogen and lipid-like vacuoles seen in the cell cytoplasm during attachment. In the argasid *O.erraticus*[33], the endospermatophore wall was broken down and sperm were released into the uterus and oviducts a few hours after 18-72 min feeding period and copulation (El-Shoura[37]). Moreover, the *O. erraticus* uterine secretion was thought to contain substances also involved in dissolving the endospermatophore wall. However, the recepataculum seminis in *H. anatolticum* must look forward for further histochemical investigations to make known its chemical nature.

The Connecting Tube

It seems no remarkable cellular activity was recorded in this organ during feeding and copulation. However, only the changes were for the expansion of the deep folds of connecting tube during oviposition. Moreover, contact of the cuticular lining with underlying cells, and microtubules associated with desmosome-like structures probably provide strong mechanical support for the wall of the connecting tube during oogenesis or egg passage. Also during oviposition, the cuticular folds disappear as the eggs pass by.

Oviducts

The paired oviducts in *H. anatolicum* combine distally to form a common oviduct. This is similar to that described in ixodid *D. Andersoni*[16], *H. Asiaticum*[17], *H. dromedrii*[32]. and H. Anatolicum[7]. The penetration of the sperm to the oviduct cell vacuoles is a

phenomenon which has been recorded for other ixodids[16,27] and argasids[14,18,33]. These vacuoles are considered to be phagolysosmes which, for unkown reason, are thought to play a role in breaking down some of the sperms ascending to the ovarian lumen. Oviducal epithelial cells respond to intercellular invasion by sperms in a clearly defensive manner. Formation of vacuolar structures, or capsules[16], apparently serves to inhibit continued intracellular movement of giving sperm cells, thus preventing further disruption of interacellular organization. The vacuolar structure then becomes a protective barrier for the host cell, increasing its chance to maintain integrity of the epithelium as suggested by Brinton *et al*[16].

The presence of lysosomal-structures in the cell cytoplasm of the oviduct they probably play a function in the breakdown of seminal fluid or other material taken up by pinoctosis during sperm passing into the lumen of the oviduct. Moreover, the dense granular secretion detected in the cell cytoplasm of the oviduct in *H. anatolicum* has been described for *D. Andersoni* [16]; their contents suggest to act as tanning agents to sclerotize the soft cuticle of the egg shell, and also possibly serving as a lubricant for egg passage[29]. However, during oviposition, the membrane infoldings dramatically increase in number and height, becoming perpendicular to the surrounding basal lamina. This kind of structure may also assist for egg passage after the oviposition.

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دراسة التركيب الدقيق للجهاز التناسلي خلال التغذية والجماع على أنثى القراد هيالوما هيالوما اناتوليكوم اناتوليكوم (اكسودويدي) (اكسوديدي)

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المملكة العربية السعودية

المستخلص: تم وصف التركيب الدقيق للجهاز التناسلي لأنثى القراد هيالوما (هيالوما) أناتوليكوم أناتيوليكوم خلال التغذية والجماع. تستهل التغيرات خلال التغذية على نمو الخلية البيضية خلال مرحلة ما قبل تكوين المح. كما أن عملية تكوين البويضات تتأثر بالتغذية. خلال نمو الخلية البيضية النامية تفرز حبيبات كثيفة في السيتوبلازم تشكل الزوائد الدقيقة السطحية. السيتوبلازم يحتوي حويصلات أجسام جولجي، الشبكة الإندوبلازمية المحببة وأجسام حويصلية متعددة، ومنطقة المهبل الذي يتركب من جزء دهليزي وجزء عنقي يتشكل من طلائية تمتد داخلياً مبطنة بواسطة طبقة من جليد ملتو وممتد، وتحاط خارجياً بواسطة طبقات من العضلات. هذه التراكيب تساعد على عبور الحيوانات المنوية خلال الأوعية المنوية، والبويضة إلى الخارج. يتركب زوج الغدد الملحقة التي تفتح عند التقاء طريق الجزء الدهليزي والجزء العنقي من المهبل من طبقة طلائية من خلايا غير منقسمة. ونتيجة للتغذية المتقدمة تبني هذه الخلايا الحبيبات الإفرازية، وذلك لتقوم بعمل ترطيب سطح البويضة خلال مرورها من الجزء الدهليزي للمهبل. الأوعية المنوية تتكون من خلايا مكعبة مبطنة من

الجليد. وهذه الخلايا تبدو غنية بالجليكوجين، تجاويف الليبيدات. والتي ربما تقوم بعمل إمداد جرعات من الطاقة التي تحتاجها الخلايا المختلفة للنشاط التي تقوم به، خاصة ببناء الحبيبات وإخراجها الخلوي. الحبيبات تخرج محتوياتها خلال الفتحات البين خلوية، وتنتشر خارجيًا خلال جدار الوعاء المنوي، تتصل بالقناة الرئيسية عن طريق قنوات ضيقة. ولا يوجد نشاط إفرازي شوهد خلال أو بعد التغذية في الأنبوب الرابط .شوهد عند وخلال مرحلة التغذية النهائية، سيتوبلازم الخلية يحتوي على تجاويف كبيرة، تم اختراقها بواسطة الحيوانات المنوية، بالإضافة إلى حويصلات دقيقة جدًا والتي تساهم في تكسير الحويصلات الحاملة للسوائل المنوية ومواد أخرى.

إفرازات قناة البيض ربما تعمل كأداة تجليد وتقوية لقشرة البويضة، وكذلك ربما تعمل كمرطب في حالة عبور البويضة للخارج. بالإضافة فإن الغشاء القاعدي المتعرج (المنثني) ربما يعطى ملامح مميزة للطلائية المعنية بنقل الماء المتأين.