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Research Article

# Histology and transmission electron microscopy (TEM) of salivary glands and gut in adult female *H. a. anatolicum* fifteen days feeding on rabbits immunized by midgut antigen.

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**Abstract**

Histological examination and TEM of female *Hyalomma anatolicum anatolicum* fed on the immunized rabbits revealed that the salivary gland showed complete destruction and degeneration in most of the three types of acini and it was difficult to distinguish between them. The cytoplasm was retracted, contained vacuoles with absence of granules. The nucleus became smaller in size and pyknosed; with irregular shape with dark condensed chromatin in the contrast to the control salivary gland. Moreover, histological changes of immunized gut of 15 day feeding of *H. a. anatolicum* revealed incomplete engorgement in compare with the same age of feeding. The lumen of gut contained smaller amount of erythrocytes with damage, degeneration and vacuoles formation in the digest cells. The TEM of immunized gut showed that, the secretory cell type 2 was ill developed. The rough endoplasmic reticulum (RER) appeared as broken down and there were many free ribosomes in the cytoplasm. The cytoplasm of digestive cell contained less haematine granules. At basal of digest cell there were large vesicles contain different lamellated body as well as myelin bodies in some heterolysosomes of digest cell. Other pathological changes were illustrated following the immunization and not found in control ticks. This study may considered to be the first time to deal with the histology and electron micrography of both salivary glands and gut of both control and immunized 15 days fed female *H. a. anatolicum* ticks.

**Keywords:** Gut, salivary gland, *H. a. anatolicum* , histology, TEM.

## Introduction

Tick infestations of man and animals are among the most significant health and economic hazards of the tropical and subtropical countries. This is especially true for Egypt **(Fahmy 1980 and 1982 and Liebisch et al., 1984).** The genus *Hyalomma* consists of 27 species **(Hoogstraal, 1981 and Liebisch et al 1984)** and is the most predominant genus of all ticks found in Egypt where 89.6% of these were *H. a. anatolicum*. Many authors studied the histological sections and histochemical of salivary glands of ixodid ticks (**Binnington,1978, Binnington & Stone, 1981, Binnington et al., 1983, Gill & Walker, 1984, Walker et al., 1985 and Nunes et al., 2005)**. Electron microscopy was done also by **Coons & Roshdy, 1973; Meredith & Kaufman, 1973; Megaw & Beadle, 1979; Fawcett et al., 1981 a& b; Krolak et al., 1982; Walker et al., 1985 and Nunes et al., 2005**. These studies indicated that, the complexity of salivary glands is not surprising since they are known to perform such varied functions as secretion of attachment cement **(Gill & Walker, 1988),** secretion of anticoagulants **(Ross, 1926 and Foggie, 1959),** a variety of enzymes **(Gill et al., 1986),** pharmacological agents that evade the host defense mechanisms **(Dickinson et al., 1976 and Ribeiro, 1985)** and elimination of excess fluid and ions during feeding **(Tatchell, 1969).** In addition, the salivary glands have been found to be the source of paralytic

toxins **(Ross, 1926)** and other components which induce immunological responses to tick feeding **(Gill et al., 1986).**

The salivary glands during the prefixation phase show massive acini, secretory cells with granules of cytoplasmic secretion and large nuclei **(Schumaker and Serra-Freire, 1991),** followed by a gradual regression with morphological and structural changes including autolysis **(Fawcett et al., 1986).** According to studies accomplished by (**Till, 1961),** as soon the engorgement phase is completed the salivary glands of ticks undergo a reduction of size (in *Argasids*) or they degenerate (in *Ixodids)*. Recently, **Nunes et al., 2005 and 2006** affirmed degenerative process in the salivary glands of females of *R. (B.) microplus* during the early feeding stage and semi-engorged feeding.
The gut of ticks is an important potential target for the defences of the host in expressing acquired resistance to ticks. There is also an increasing interest in the use of vaccination against ticks based on the use of antigens derived from the gut **(Agbede & Kemp, 1986; Johnston et al., 1986 and Kemp et al., 1986).** Midgut digestive cells are a place for storing proteins, lipids and carbohydrates and may also be involved in intermediate metabolism **(Balashov, 1962).**The main studies of the structure and function of tick gut cover *Ixodes* ticks, were by **Hughes (1954),** *Haemaphysalis spinigera* by **(Chinery ,1964),** *R. appendiculatus* by (**Till, 1961**), *Hyalomma asiaticum* by **(Balashov ,1983 , Balashov & Raikhel ,1976 and Raikhel ,1974, 1975 and 1978)** and *Boophilus microplus* were by **(Agbede & Kemp,1985).** To study the effects of host resistance on the gut of the tick it was found necessary to have a detailed description of the ultrastructure of the species used **(Walker and Fletcher, 1987**).

**Gill and Walker, 1984,1987 and 1988** described the sequence of structural changes occurring in the salivary glands of both male and female *H .a. anatolicum* ticks during feeding and the nature of salivary components and their role in feeding and **Bughdadi, 2010 and 2012** studied the ultrastructural of muscles fibers and fat body cells in nymph of *H. a. anatolicum.*

Considering that rare studies were specifically focused on the structural changes that happened on salivary gland and midgut of adult female *H. a. anatolicum* 15 day feeding on immunized rabbits and control, the aim of the present study was to describe the structural changes occurring on the salivary gland and gut of *H. a. anatolicum* 15 day feeding on rabbit (no previous exposure to ticks) and immunized rabbits by midgut antigen.

## Materials and Methods

### Tick colony

*H. a. anatolicum* ticks used in this study were obtained from a colony maintained at the Department of parasitology, Faculty of veterinary medicine, Cairo University, Giza. Ticks were collected from naturally infested cattle in Baharia, Al Kharga and Siwa Oases in Egypt. They were maintained at 28°C and 85% relative humidity. Both male and female ticks were fed as described by **Walker et al., 1985** on the ears of New Zealand white rabbits, which had no previous contact with ticks, mites were coccidia free. Partially fed (4 to 5 day old) *H. a. anatolicum* adult females were used for preparation of midgut antigens. The midgut antigen was prepared using the method of **(Wong and Opdebeeck,1989).**

### Antigen preparation

The midgut were dissected out and washed with PBS (PH 7.2) to remove host blood contents. The midgut were homgenized by homgeizer for 5 times, each 2 minutes with one miunte cooling intervals in ice bath, then these homginized tissues were sonicated by ultrasonicator at 100W standard probe for 5 times, each 2minutes with one minute cooling intervals in ice bath. Sonicated tissues were centrifuged by high speed cool centrifuge at 14000 rpm for one hour at 4 °c **(Heller-Haupt et al., 1996).**

Each supernatant containing antigenic material were separated. The protein content of the supernatant containing antigen material was measured by the method of **(Lowry et al., 1951).** The antigen was aliquot and stored at -70° c until use.

### Immunization Schedule

Ten Male New Zealand white Rabbits each of 2 -2.5 Kg body weight were kept and bred according to the role of EAURC (Ethic of animal use in research committee) with code number 2014/34.The rabbits were divided into two even experiments groups

(immunized & control). Rabbits were immunized against *H. a. anatolicum* ticks according to the protocol of artificial immunization mentioned by(**Kumar et al., 2002**) using midgut antigen.

Primary immunization was done on day Zero, by injection of five rabbits with 350µg of midgut antigen emulsified with mineral oil in equal volume (1ml) intramuscular on each side of the back. 1st booster was done on day 14th, by injecting 175µg protein from midgut antigen emulsified with mineral oil in equal volume (1ml) S/C on shoulder sides. 2nd booster was done on day 28th (as 1st booster) on back.

**Challenge infestations**

The challenge was done on day 35 (7 days post 2nd booster) on ear by using ear bag with 20 adult male and female ticks on each animal. Ticks were collected from rabbits after completely detached full engorged adult female from control animals, while other ticks still attached to resistant rabbits and became partially engorged. So the ticks were collected and detached after 15 days. Twenty adult female ticks 15 days feeding challenged from both immunized and control groups were dissected for obtaining salivary gland and gut.

Dissection of 40 adult females (20 females for histology and other 20 adult female for TEM) was done to collect gut and salivary glands according to **(Gill et al., 1986)** where the adult female ticks were washed in saline, fixed in wax plates and opened alive using fine needle, forceps and scalpels under binocular dissecting Olympus microscope. Ticks were washed with cold PBS (Phosphate buffer saline PH 7.2).

**Histology**

The salivary glands and gut were fixed in v/v, 9:1, mixture of neutral buffered formalin and acetone10% (pH 7–7.4) at 48°C for 2 h, and then dehydrated in ethanol Series (70- 80-90-95%) at 15 minute intervals. The inclusion was performed in Leica resin and the polymerized blocks were sectioned with a dry glass knife. The sections of (3-5 µm) thickness were put on cleaned glass slides. They were air dried before stained with Hematoxylin and Eosin. **(Junqueira and Junqueira, 1983)** and examined and photographed in a Olympus-BX 15 photomicroscope.

**Transmission Electron Microscopy (TEM)**

The salivary glands and gut were fixed in 2.5% glutaraldehyde, kept for 4 hr at 4°c, thoroughly rinsed over night at 4°c in 0.1 Mphosphate buffer, postfixed in 1% OsO4 for 30 minute at 4°c, then washed in PBS (PH7.3) for 1 hour followed by dehydration in a grad series of ethanol (50%-90% for 15 minute and 100% for 1 hour). After that, they were then routinely processed and embedded in epon Araldite **(Spurr, 1969)**. Semi-Thin sections (0.5 micron) were cut with a glass knife using a RMC, Inc. microtome and collected on copper grids then stained with Toluidine blue 1% and in McIlvane buffer (pH 4.0) for 20 min, washed in distilled water and set in Permount. The obtained slides obtained were analyzed and photographed using an Olympus-BX 15 photomicroscope. **(Nunes et al., 2006).** Ultra-thin sections were stained for 15 minutes with a saturated urenyl acetate solution and counter stained in lead citrate for 20 minutes (thickness 500-700 angstrom); **Reynolds, 1963.** Grids containing ultrathin sections of the material were analyzed and photographed in a Joel JEM-1200 EX II transmission electron microscope (Electron Microscope unit, in Military veterinary Hospital in Nasar city.

## Results

**Histology of salivary gland**

The three types of acini in salivary glands of final stage of feeding *H. a. antolicum* females (15 days) fed on control and immunized rabbits were identified. Differentiation between control and immunized salivary gland were done through showing degree of degeneration at the same age of feeding by which the salivary gland normally starts in degeneration at early stage of feeding and semi-engorged ticks.

Type I acini showed slightl features of cell degeneration in final stage of feeding in control one Fig 1(A, B, C and D). The cell's cytoplasm appeared with fine granulation and vacuolation. The nuclei located at periphery and with non condense chromatin. The acini type II and III showed degree of normal degeneration so as it was difficult to differentiate between the type of cells of acini type II and III where nucleus was condense and irregular and fragmented, in addition to extensive cytoplasmic retraction and vacuolization with low number of granules Fig 1(D). On other hand, the same age of salivary glands of

immunized tick showed complete destruction and degeneration in most of the three types of acini and it was difficult to distinguish between them. The cytoplasm contained vacuoles, retracted with absence of granules. The nucleus became small sized and pyknosed; started to disappear, irregular in shape having dark condensed chromatin Fig 1 (E&F).

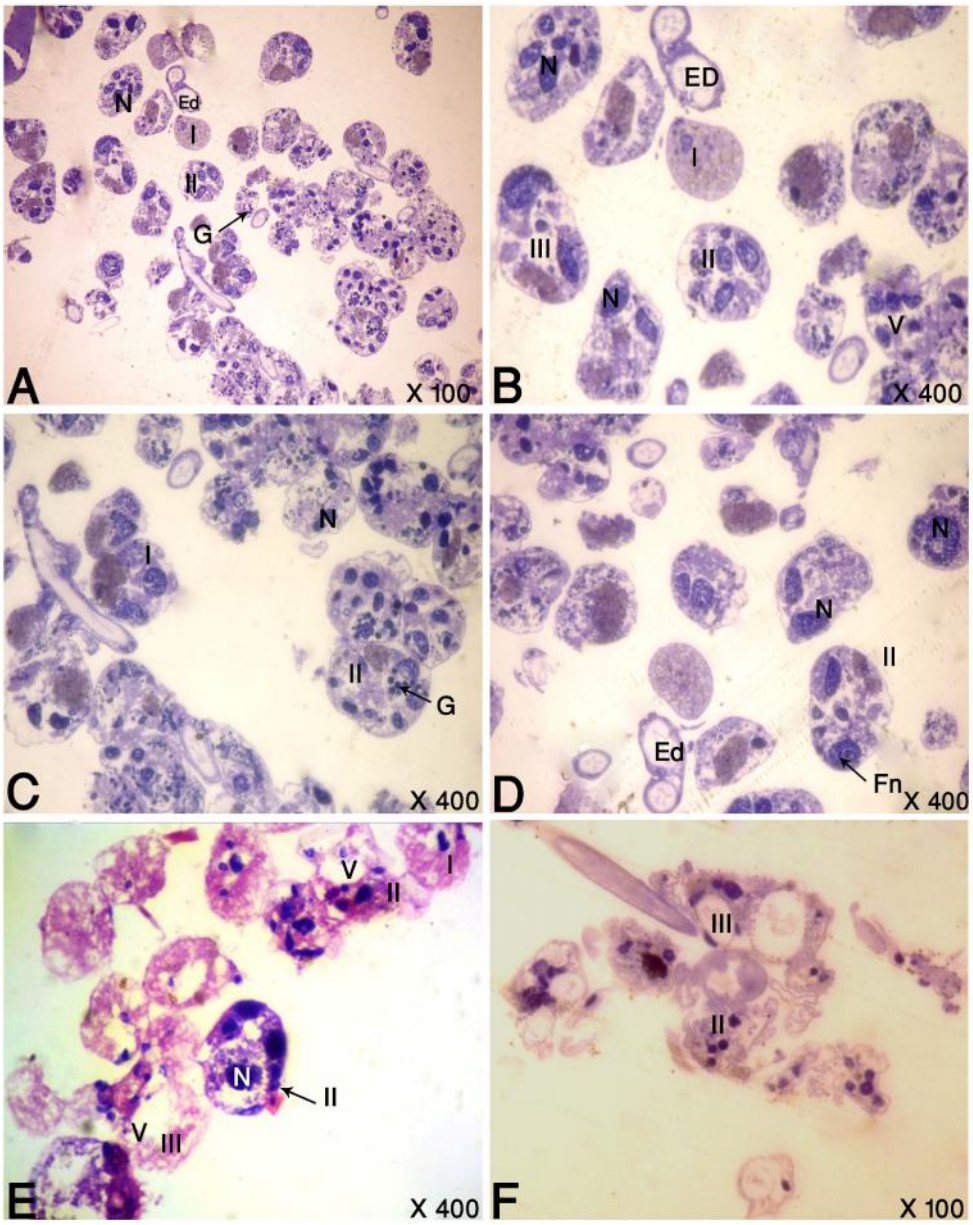


Fig (1): Histological sections of the salivary glands of females *H. a. anatolicum* at age 15 days feeding on control and immunized rabbits. In control salivary gland stained with toluidine blue showing degeneration of three types of acini, exhibit extensive cytoplasmic retraction areas and intensive vacuolization and consequence decreased number of granules. The cells were with irregular nuclei, condense chromatin and start fragmentation process. (A, B, C and D) In immunized salivary gland stained with H&E (E) and toluidine blue (F ): severe destruction and degeneration in most of the three types of acini and difficult to differentiate between them, small condense and pyknosed nucleus and retraction of cytoplasm and vacuolization with absence of granules.

Ed excretory duct, I acini type I, II acini type 2, III acini type 3, V vacuole, Fn fragmented nucleus, N nucleus, G granules

**Transmission electron microscopy of salivary gland**

The electron microscopy of salivary gland tissue of control ticks showed degeneration but with less severity than that of the ones immunized at the same age of feeding. The acini type II of control salivary gland showed slight vacuolation in the cytoplasm Fig 2. The number of granules were more than that in immunized salivary gland. There were different types of granules having different shape, size and structure (G1, G2& G3). Some granules began to degenerate and change in the structure into remnant of granules and granules with electron lucent vesicles at margin. Other granules start to show vacuoles inside them. The mitochondria and Golgi apparatus appeared smaller in size and degenerated. Moreover, the nucleus appeared slightly smaller in size. Some cells contain nuclei with pyknosis. Fig 2 (A, B, C, D, E &F).

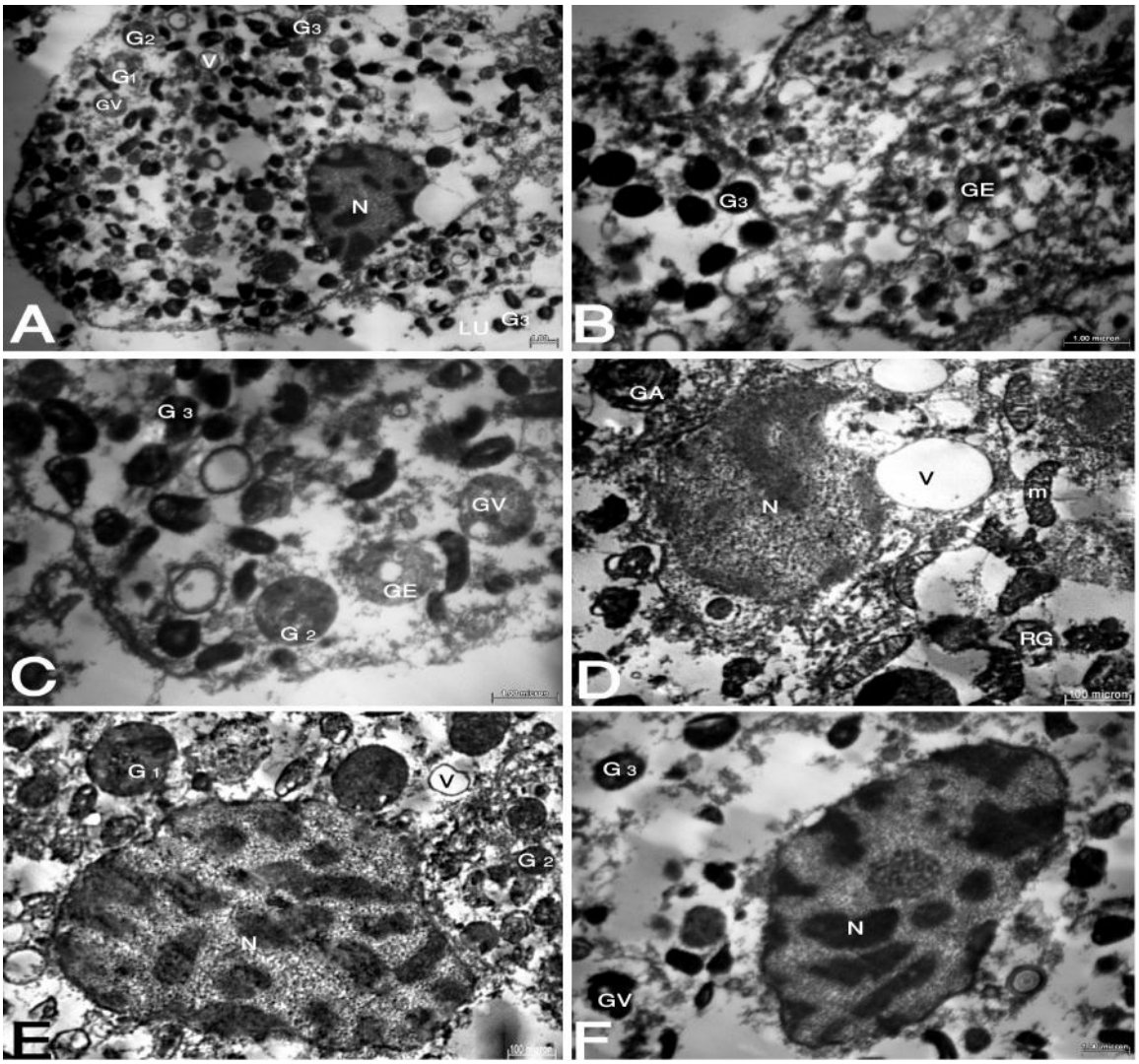


Fig (2):Electron micrography of salivary gland of control female tick showing the cells of acini type II, the cytoplasm had different vacuoles and large number of granules of different shape , size and structure ( G1, G2 &G3). Some granules start to change in structure into reminant of granules and contain vacuoles. the mitochondria and Golgi apparatus start to degenerate. Notice the pyknosis of nucleus and chormatin slightly reguralar arrangement (A, B , C, D, E&F).

G1, G2 & G3 different types of granules, RG reminant of granules, GV granules with vacuoles, GE granules with electron lucent vesicles, V vacuoles, LU lumen, GA golgi apparatus, m Mitochondria and N nucleus.

Regarding to the immunized salivary gland acini type II showed less number and smaller sized granules. These granules became degenerated, others contained vacuoles. The cytoplasm was completely degenerate and destructed. The RER (rough endoplasmic reticulum) degenerated and left remnant of ribosome free in the cytoplasm. Golgi apparatus showed complete degeneration and destruction. Furthermore, the nucleus of cells of acini type II appeared pyknosed, small in size and of different shapes. The chromatin appeared very dense and dark in colour with presence of multiple vacuoles inside the nucleus. The nuclear membrane showed degeneration Fig 3 (A, B, C& D).

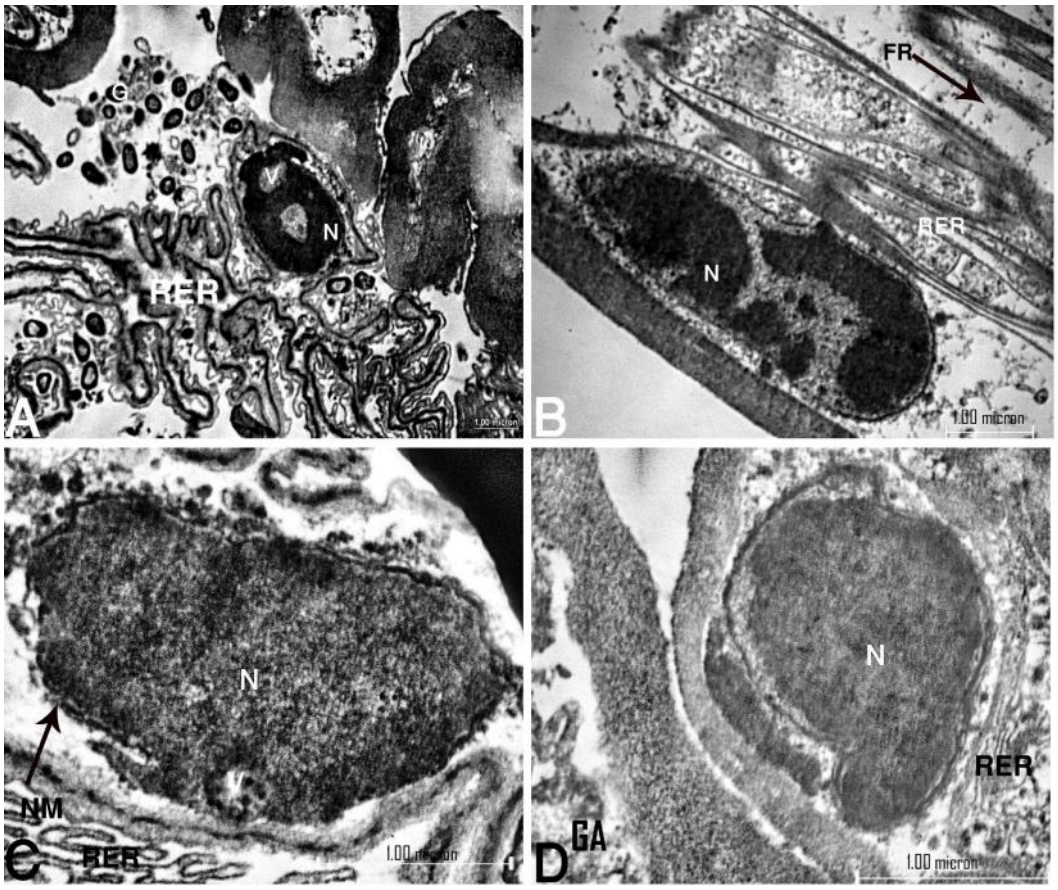


Fig (3):Electron micrography of salivary gland of immunized female tick showing the cells of acini type II, appear low number of granules and small size and contain vacuolesThe rough endoplasmic reticulom (RER) destructed and left ribosome free in cytoplasm. The cytoplasm degenerated and retracted . Nucleous (N) became condense of chromatin arrangement and contain vacuoles. The nuclear membrane began to degenerate and destruct (A, B, C &D).

G granules, RER rough endoplasmic reticulom, FR free ribosome, GA Golgi apparatus, vacuoles, N nucleus and NM nuclear membrane.

**Histology of gut**

The ticks which had fed for 15 days on control rabbits, appeared normal and their gut were lined with intact digestive cells and secretory cells. The lumina did not contain cell debris or host leucocytes but completely filled with Rbcs. Fig 4 (A&B). The light microscopy showed that, the cellular organization of the gut epithelium is complex and composed of different cell types. One of these, the digest cell, developed through series of stage to mature cell which filled with host cells and fluid taken in gut from gut lumen by pinocytosis and phagocytosis. Moreover, there are three generation of these digest cells during feeding of female ticks. There are two secretory cell types (Sc1& Sc2) present during feeding and some digest cell slaughed in lumen of gut. This digest cell may contain most of the haemoglobin as cell lysis has occurred in the lumen. A range in size of the digest cells could reflect the different ages of the cells. In the fully

engorged ticks the gut stretch, such that in places, only a thin layer of flattened cells line the gut lumen. Secretory cell type 1 (Sc1) had columnar to cuboidal shape. The nucleus occupied the central part of the Sc1 cell, and the cytoplasm was packed with homogenous densely staining round granules. The Sc 2 cell occurred singly or in groups of 2 to 3 cells, interspersed between the digest cells, and were not confined to any particular region of the gut. Delayed development of gut of ticks fed 15 day on immunized rabbits was indicated by incomplete engorgement with the same age of feeding. the midgut did not reach the size necessary for the consumption of the blood bulk sufficient for the morpho-functional reconstructions of the midgut itself and also for the final development and functioning of salivary glands and for egg development. The lumen of gut contained smaller amount of erythrocytes with the presence of damaged leukocytes. Segeneration and vacuoles formation were detected in the digest cells Fig 4 (C,D,E&F).

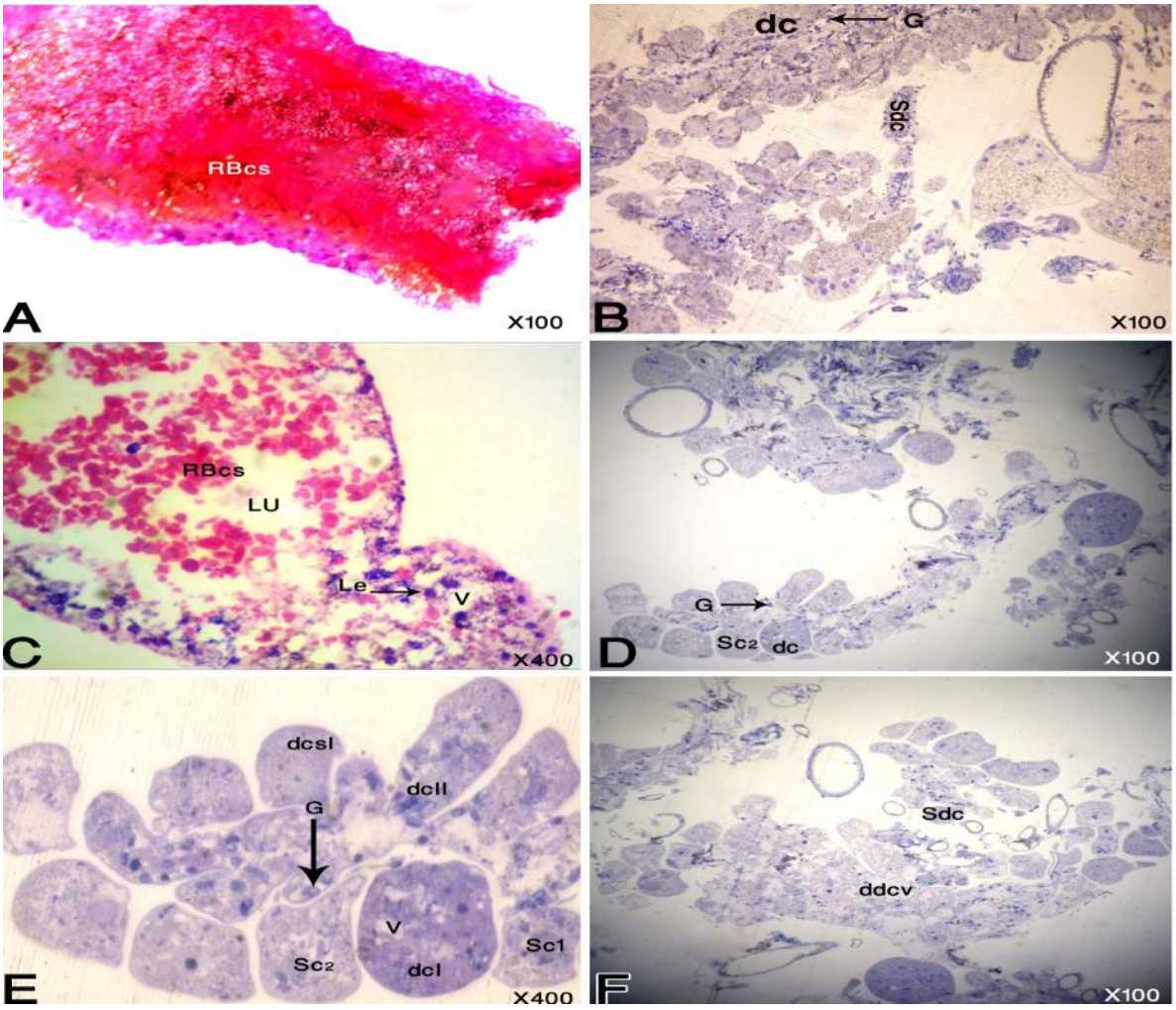


Fig (4): Histology of gut of female *H. a. anatolicum* after 15 days feeding. The gut of control ticks stained with H&E (A) showed engorgement of the lumen with Rbcs . The digest cell appeared normal in appearance and contain large number of granules (B). The gut of females immunized tick at the age of feeding showed distended lumen, small amount of Rbcs, leukocytes and completely destroyed digest cell and secretory cell Stained H&E (C). The gut stained with toluidine blue (D,E&F) showed degeneration of digest cell with vacuoles in the cytoplasm and low number of granules (C,D,E&F).

dc digest cell , dcI digest cell type I, dcII digestive cell type II, dcsI digestive cell subtype I, ddcv degenerated digest cells with vacuoles, Le Leucocytes, Sc1 Secretory cell type 1, Sc2 secretory cell type II, N nucleus, V vacuoles, Sdc slaughed digest cell, LU lumen, G granules and RBcs erythrocytes.

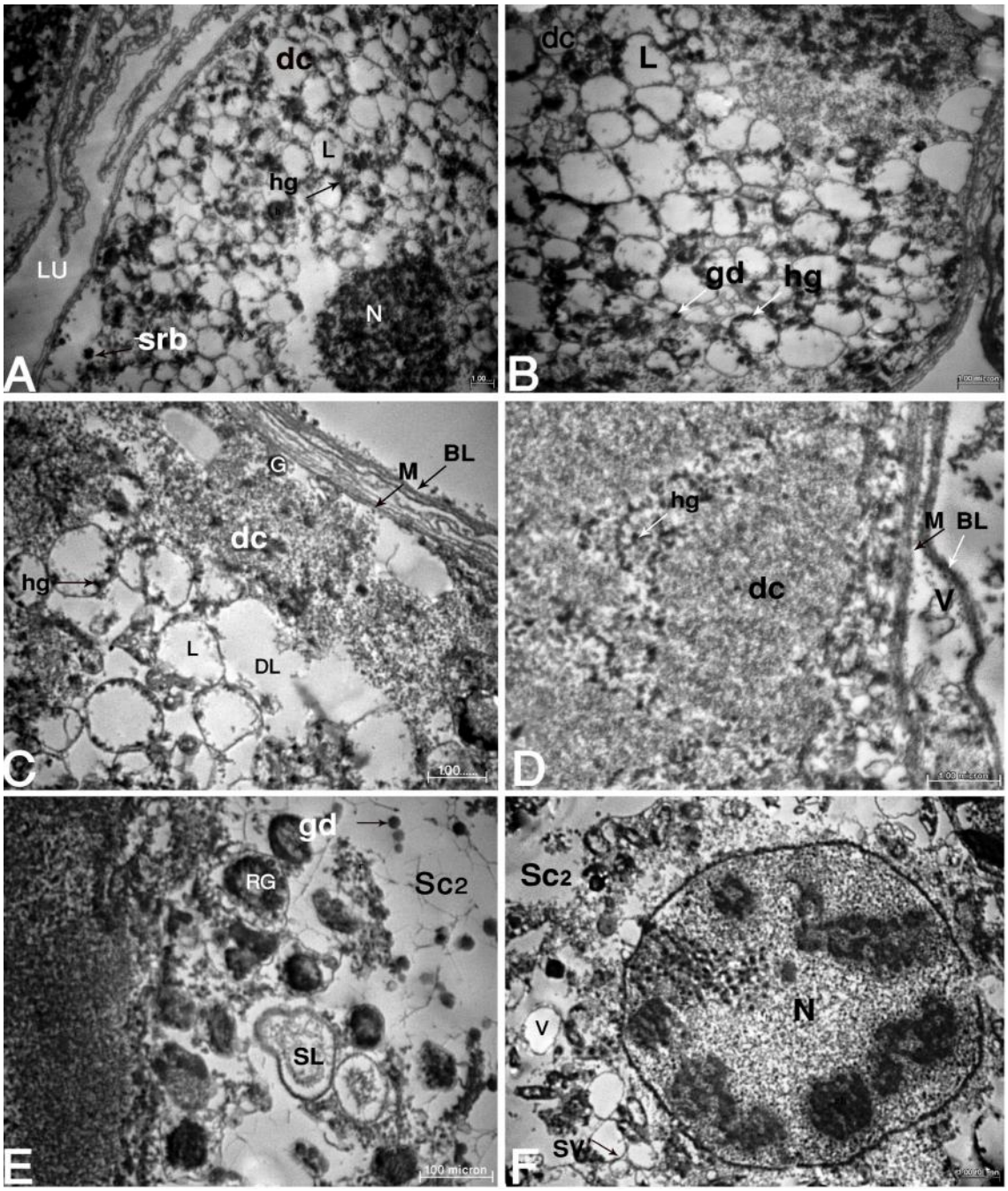


Fig (5): Electron micrograph of gut of control female ticks fully engorgement showing normal appearance of digest cell. The cytoplasm of digestive cell contained different type of granules, large lipid like granules with haematine granule, glycogen deposite inbetween lipid granules and secretory lysosome. The nucleus appeared slightly normal and regular arranged chromatin.

L lipid like granules, DL degenerated lipid like granules, dc digest cell, V vacuoles, hg haematine granules, N nucleus, gd glycogen deposite, M muscles, BL basal lamina, SV secretory and excretory vesicules, LU lumen , G granules, Srb sessile residual body, SL secretory lysosome ,RG reminent granules and Sc2 secretory cell type II.

**Transmission electron microscopy of gut**

The gut of female *H. a. anatolicum* fed 15 day on control rabbit which were used for electron microscopy had maturing secretory cells of type 2 (Sc2). After engorgement, reorganization occurred but the origin of the cells could still be traced because some of them had a few remaining Sc2 granules. The cells further enlarged and extended towards the lumen, thus maintaining contact with digest cells were also expanding into the lumen as they ingested more of the blood meal. The cytoplasme of secretory cell type 2 contained lipid like granules with large number of haematine granules. Large lipid-like granules and glycogen deposits formed accumulations above and below nucleus and have present haematine granules.

In addition, the digest cell type I was intact, had large number of granules and lipid like granules appeared normal intact and start to be enlarged. Some lipid like granules were fused as large vacuoles. The nuclei appeared slightly normal with regular arrangement of chromatin.

In gut of immunized ticks the cytoplasm of Sc2 had the profile of some granules included clusters of vesicles which, in some cases, had a protubrance that extended into cytoplasm. The cytoplasm of digestive cell contained less haematine granules. In ill developed secretory cell type 2, the RER appeared to be break down and there were many free ribosome in the cytoplasm (Fig 7,K). The granules became closely associated with the lipid and appeared to be coated with an uneven layer of electron-lucent material on the outer surface (Fig 6, E).

The digest cell had low number of lipid like granules. Other granules had different degree of degeneration such as remnant granules, granules contain vacuoles, granules with pointer border and granules with dense body at margin. Other granules have been illustrated in Fig 6 and 7.

At basal of digest cell, large vesicles contain different lamellated body and myelin bodies in some heterolysosomes of digest cell were noticed Fig 7 (F, L& M). The nucleus showed arrangement of chromatin materials at the periphery and start to decrease in size. Also there were vacuoles in the center and peripherial of the nucleus Fig 7(K&L). The cytoplasme showed multiple vacuoles. The digest cells appeared with pyknotic nuclei with vacuoles in cytoplasm. Heterolysosomes were homogenous containing pinocytotic material in sessile and motile digestive cell(Fig 7 A&J).

**Discussion**

There are three types of acini in salivary gland of final stage of feeding females *H. a. anatolicum* (15 days) fed on control and immunized rabbits which is true for the general organization in Ixodid ticks **( Till, 1961, Chinery, 1965, Coons &Roshdy, 1973, Binnington, 1978, Krolak et al ., 1982, Walker et al., 1985 and Gill & Walker, 1987**) with the exception of female *Haemaphysalis leachi***,** which contains one agranular and three granular acini types (**El Shoura, 1985**). Histological description of salivary gland of females *H. a. anatolicum* 15days feeding cleared that, Type I acini showed slight cell degeneration in final stage of feeding in control one. The cell's cytoplasm presented fine granulations and vacuoles. The nuclei located at periphery with non condense chromatin. The acini type II, III showed degree of normal degeneration where nucleus was condense, irregular and started to fragment with extensive cytoplasmic retraction and vacuolization with low number of granules. The salivary glands of immunized ticks showed complete destruction and degeneration in most of the three types of acini and it was difficult to distinct between them. The cytoplasm contained vacuoles with retracted and absence of granules. The nucleus became pyknosed, small sized, irregular in shape with dark condense chromatin. This study might considered to be the first record for histological changes of salivary gland of immunized and control females *H. a. anatolicum* 15 days feeding. As mentioned by **Fawcett et al., 1986** and **Schumaker and Serra-Freire, 1991**; the salivary gland presented functional and structural changes which were determined by the physiological stage of the tick. They cleared also that, secretory cells with granules of cytoplasmic secretion and large nuclei followed by a gradual regression with morphological and structural changes including autolysis were occurred.

According to studies accomplished by **Till , 1961,** as soon the engorgement phase is completed the salivary glands of ticks undergo a reduction of size (in *Argasids*) or they degenerate (in *Ixodids)*. Recently, **Nunes et al., 2005 and 2006** affirmed that salivary glands of *R. (B.) microplus* female ticks already show

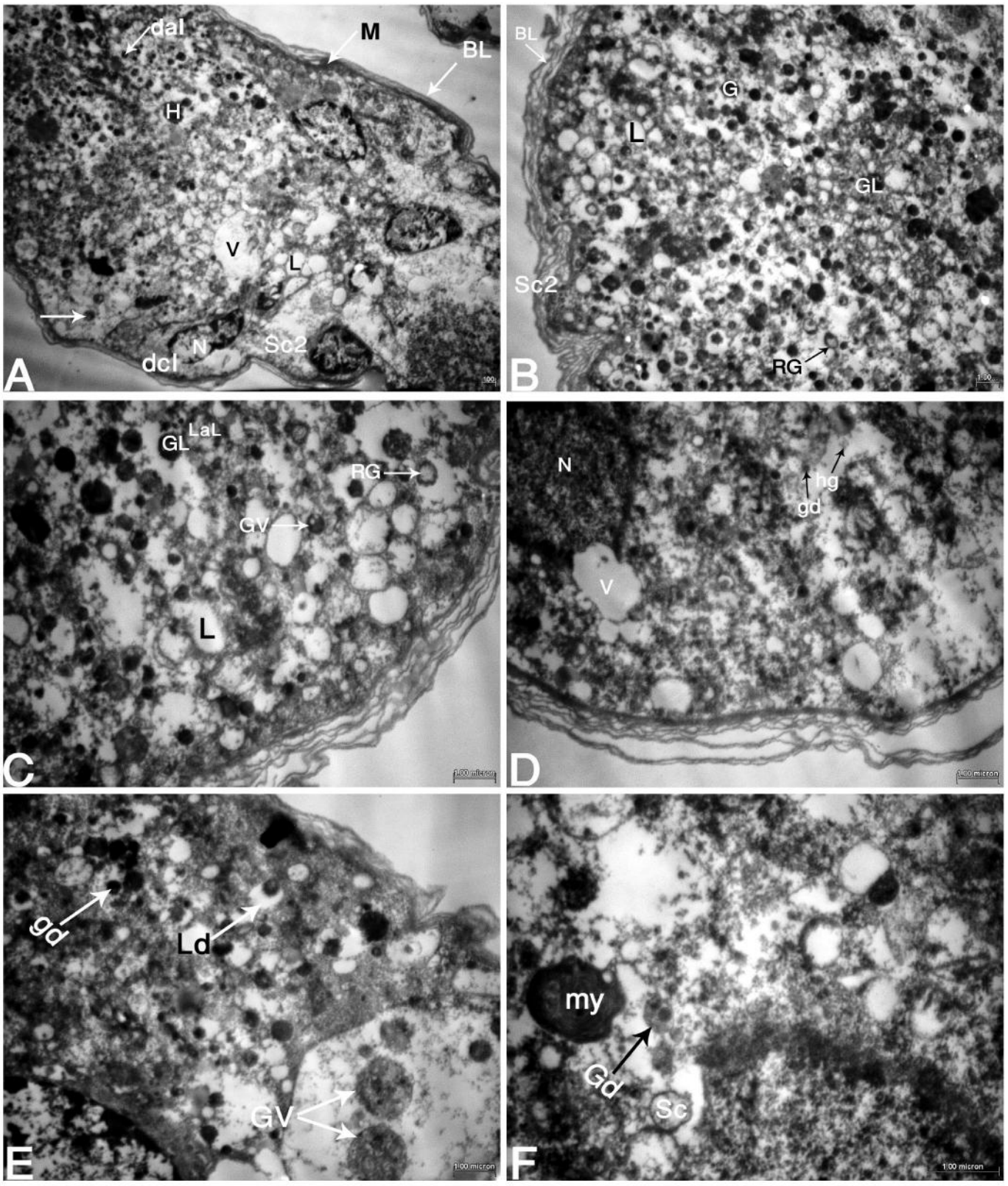
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Fig (6)

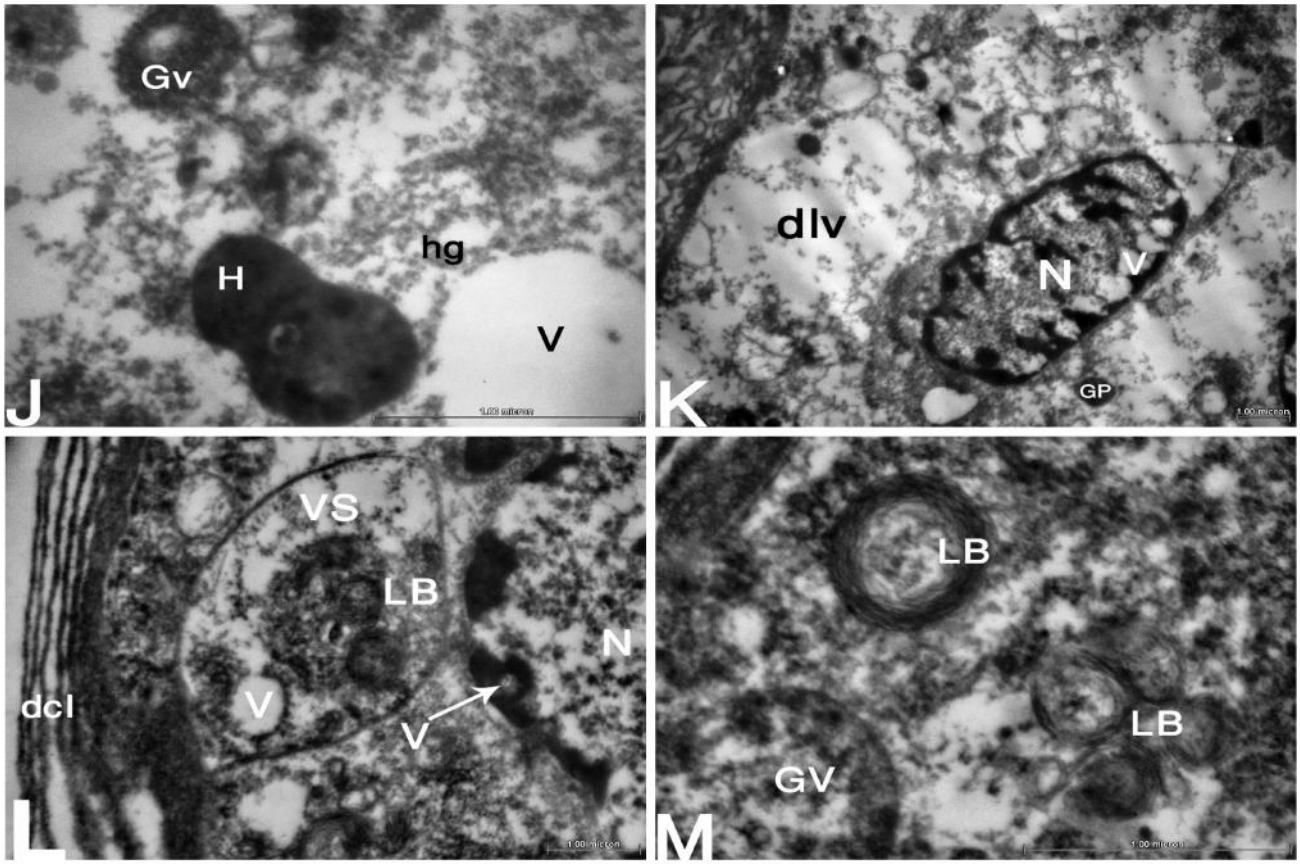


Fig (7)

Fig (6&7): Electron micrograph of gut of immunized ticks showing atrophy of digest cells and secretory cell (A). The secretory cell type 2 showing low number of haematine granules (B). Destruction of lipid like granules (C&D). The other granules in motile digestive cell appear granules with electron lucent vesicles at other granules with pointed border in secretory cell type 2 (B, C, D, J&K). Heterolysosomes with homogenous contain pinocytotic material in sessile and motile digestive cell (A&J). Different lamellated bodyand myelin body in heterolysosome appeared in the cytoplasm of digestive cell (F,L&M). The nucleus showed arrangement of chromatin materials at the periphery. Also there were vacuoles in center and peripherial of the nucleus (K&L).

L lipid like granules, dlv degenerated lipid like granules with vacuoles, Ld lipid like granules with dense granules , dcI digest cell type I, rsd residual sessile digested cell, dal dense autolysosome, LaL laucent autolysosome, Sc2 secretory cell II, Sc secretory lysosome, H heterolysosome, V vacuoles, hg haematine granules, N nucleus, G granules, GP granules with pointer border, gd glycogen deposite, Gd granules with dense body at margine, M muscles, BL basal lamina, GL granules with electron leucent vesicles, GV granules with electron lucent vesicles on centeral and border, RG reminant granules, LB lamellated body, my myelin body and VS vesicles.

a degenerative process at early feeding stage and the semi-engorged stage. All this claims supported these studies.

The ultrastructure of salivary gland tissues of control ticks showed degeneration but with less severity than immunized salivary gland at the same age of feeding. The acini type II of control salivary gland showed slightly vacuoles in the cytoplasm, the number of granules were more than in immunized salivary gland. The types of the described granules in acini type II agree with finding of **Megaw and Beadle, 1979** who studied salivary glands of *B. microplus*. Moreover, in the immunized salivary glands some granules began to degenerate and change in the structure into reminant of granules and granules with electron lucent vesicles at margin. Other granules started to show vacuoles inside them. The mitochondria and golgi apparatus appear smaller in size and degenerated. Nucleus appeared slightly smaller in size. Some cells contain nuclei with pyknosis.

According to **Kerr et al., 1995** (*B. microlpus*), **Sauer et al., 2000 (***B. microlpus* **), Denardi, 2002** *(Amblyomma cajennense*), **Oliveira, 2002 (***R. sanguineus)* and **Nunes**; **et al 2005** (*B. microlpus*), the TEM technique provided the evidence for the presence of nucleolar remains inside the nucleus when apoptosis occurred. These appeared either as rounded structures of low electron-density or as compact and rounded masses of high electron-density, frequently located next to the peripheral chromatin. Therefore, these data allow to suggest that the salivary glands of semi-engorged female ticks were already in an advanced stage of degeneration. Also, organelles as mitochondria showed a disorganized internal structure while ribosomes and the vesicular rough RER maintained a reasonably normal morphology yet. **Bowen and Bowen, 1990** added that, the histological, histochemical and ultrastructural analyzes of the secretory cells of the salivary glands of *R. (Boophilus) microplus* showed intense cytoplasmic vacuolization; the initial formation process of these vacuoles is closely related to the loss of cytoplasm caused by the degeneration.

This study considered to be the first record for TEM changes of the immunized salivary gland (acini type II) which proved less number, smaller sized and degenerated granules with vacuoles. The cytoplasm, RER and golgi apparatus showed complete degeneration and destruction. Furthermore, the nuclei were pyknosed small and different in shape with degenerated nuclear membrane.

This claim in the present study, is supported by the high percentage of gut-damaged females on immunized rabbits compared with the controls. The gut-damaged ticks differed from control ticks, on immunized animals, the damaged ticks in the final stages of feeding had sever degenerated gut. When gut damage was more extensive, low amount of Rbcs in lumen contents was noticed. The histopathology picture in the gut indicated that there could also be a contribution from host Rbcs. The ticks fed on immunized rabbits contained less amount of Rbcs in the gut compared to controls, and from the observations on degeneration of digest cell associated with vacuoles and damage, it is suggested that, these reactions played some role in the success of vaccination. Once the primary lesion had formed in the gut, reactions against other tissues which are antigenic (salivary gland etc.) could occur and these might also be important. Moreove, gut damage could affect oviposition in females by stopping digestion or blocking synthesis of vitellogenesis by gut cells.

The delayed development of gut of ticks fed 15 days on immunized rabbits that was indicated by incomplete engorgement with the same age of feeding, smaller amount of erythrocytes, degeneration and vacuoles formation in the digest cells came nearly in agreement with **Walker and Fletcher , 1987** who described the histology of gut of feeding nymph of *R. (Boophilus) microplus* found nearly the same description. Moreover, **Agbede and Kemp, 1986** studied the histological of gut of *R. (Boophilus) microplus* adult as indicated nearly the same description. **Grigoryeva, 2009** studied the morpho-function of gut cells during the life cycle (larva, nymph and adult) of *Ixodes species*.

Transmission electron microscopy of gut of female *H. a. anatolicum* fed 15 day on control rabbit observed secretory cells of type 2, the cells enlarged and extended towards the lumen, thus maintaing contact with digest cells. The cytoplasm of secretory cell type 2 contained lipid like granules with large number of haematine granules. Large lipid-like granules and glycogen deposits formed accumulations above and below nucleus and have present haematine granules. In addition, the digest cell type I showed intact, had large number of granules and intact enlarged lipid like granules. The nuclei appeared slightly normal with regular arrangement of chromatin. On the contrary, in the gut of immunized ticks, the cytoplasm of Sc2 had the profile of some granules which became closely associated with the lipid and appeared to be coated with an uneven layer of electon-lucent material. The RER damaged with many free ribosomes. These criteria with the less haematine granules indicated the immaturity of these secretory cells. The electron microscopy of gut of nymph of *R. appendiculatus* fed on resistant animals showed that, all stem cells were affected. Their nuclei were poorly defined and poorly stained and without intact nuclear membrane. The RER was poorly defined with swollen mitochondria. Secretory cells and motile digestive cells showed few pathological changes with heterolysosomes derived from the phagocytosis of erythrocytes, neutrophils and eosinophils (**Walker and Fletcher, 1987**).

The digest cell had low number of lipid like granules. Other granules had different degree of degenerations. At basal of digest cell appeared large vesicles contain

different lamellated body. The nucleus showed pyknosis, the arrangement of chromatin materials at the periphery decreased in size and vacuoles in center and periphery were noticed. The cytoplasme showed multiple vacuoles. Heterolysosomes were homogenous containing pinocytotic material in sessile and motile digestive cell and some heterolysosome contained myelin bodies. These descriptions came in agreement with finding of melanin (myelin bodies) which mentioned by **Hughes, 1954** (*Ixodes ricks* adult), **Walker and Fletcher, 1987** (*R. appendiulatus* nymph) and **Agbede and Kemp, 1986** (*B. microplus* adult).

Conclusion: Histological and TEM examination of female *H. a. anatolicum* 15 days fed on rabbits immunized showed that, the salivary gland indicated complete destruction and degeneration in most of the three types of acini and there were difficult to distinct between them. Immunized gut revealed incomplete engorgement with degeneration of gut cells in compare with the same age of feeding. Inaddition, other pathological changes illustrated sucesseding the immunization and not found in control ticks.

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