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# Study on mosquito ovary using transmission electron microscopy

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**Abstract**
The study focuses on the ovaries of mosquito using transmission electron microscopy. Mosquito that were used comprised of six different strains, namely: *Culex pipiens* Complex, *Aedes aegypti*, *Aedes vexans*, *Ochlerotatus cantans*, *Ochlerotatus rusticus* collected from the Philippines and Germany. A meal of blood in the mosquito can alter a series of changes in the cell structure that leads to the formation of a mature egg. After the mosquito has taken a blood meal, some prominent changes occur in the epithelial follicles. The very prominent changes that occur are the development of large inter-cellular spaces and a decrease in desmosomal connections. Several numbers of microvilli covered the area of the oocyte facing the epithelial follicles of which during seven hour after blood meal, push towards the extra-cellular spaces created by the separation of the epithelial follicle cells. The microvilli vary in length and are not regularly distributed like those of the typical intestinal epithelium. A unit membrane is seen which later fuses to form a yolk. A meal of blood or controlled diet leads to a series of changes in cell structures in the reproductive organ of female mosquitoes that quickly results to the formation of matured eggs. Another prominent changes observed in the ovary of mosquitoes having blood meal is the presence of various un-oriented microvilli in the area of the oocyte which is adjacent to the epithelial follicles. These microvilli can also be observed in ovary of mosquitoes having no blood meal but they are smaller in size. Furthermore, pits or vesicular bodies are developed after a mosquito has taken a blood meal.

**Keywords:** mosquito, ovary, oogenesis, transmission electron microscopy

## Introduction
Most of the insects including mosquitoes reproduce sexually. Adult mosquitoes, typically obtain nutrients for survival and reproduction from three sources [1, 2]: (1) teneral reserves from larval feeding on the microbiota and detritus in water, (2) nectar or other plant juices for energy, and (3) blood taken only by females for egg production.
Mosquito ovaries are classified as meroistic type because they contain nurse cells as well as oocytes. Furthermore, they are categorized as polytrophic because groups of nurse cells are enclosed with an oocyte in each ovarian follicle. In contrast to other insects, like for instance mayflies (Ephemeroptera) and cockroaches (Blattodea), no nurse cells are present within the oocytes. This ovary is classified as telotrophic meroistic type. In these insects, the follicle cells are equipped to carry out secretion and protein synthesis [3].
A central chamber or calyx is present in each paired ovary. The calyx is lined by an epithelium which contains muscle filaments that are also present in the lateral oviduct. Two tracheae enter each ovary and branch into the tracheoles within the ovarioles. Each paired ovary consists of variable numbers of tubular epithelial ovarioles where the oocytes are placed in a linear sequence according to their stage of growth [3].
The number of ovarioles ranges from 50 to 500 depending on the species, physiological stages as well as the size of the individual female [1]. Each of the ovariole is enclosed by a sheath which consists of cells forming a thin squamous mesothelium. Proximally, the ovarian sheath forms the long suspensory ligament which inserts into the 4$^{th}$ abdominal tergite.
The ovariole contains a germarium as well as a maturing and presumptive follicle. In the anterior part of each ovariole, are the differentiated cells of germarium through which the mitotic divisions of germ cells takes place resulting in the formation of the oocytes. In mosquitoes, these are accompanied by other cells which later perform the task of nursing or supplying nutrients to the developing gametes. In other insects, however, special nurse cells are absent and instead the nutritional responsiblity falls upon the epithelium of the oocyte follicles [3]. In the posterior portion of the ovariole, approaching to the oviduct, lies the

vitellarium which is composed of increasingly large and advanced oocytes, each is located within a follicular sac. When the oocyte is fully mature, it passes into the oviduct. Each of the follicle contains seven nurse cells and one oocyte within the follicular epithelium and the youngest follicle cells "oogonia" lie in the distal position [4]. The germarium, nurse cells, follicles and follicular stalks which compose each ovariole are invested by a basal lamina, which is sometimes called tunica propria [5, 6].

The common oviduct as well as the posterior parts of the lateral oviducts and most of the accessory glands are ectodermal in origin, whereas the real reproductive organ, the ovaries, are of mesodermal origin. The ovaries develop from a special section of splanchnic mesoderm, which is called the genital ridge. This ridge bears the germ line cells, which are sometimes segmentally separated into smaller groups.

Female mosquitoes have various sources of food during their life period. For the regular metabolism, they are feeding on flowers and fruits as a source of energy (e.g. for flying). However, for the development of eggs, they usually need a blood meal as a source of protein. The physiological and structural changes that occur after having a blood meal are still under intensive investigations.

## Materials and Methods

The study was conducted at the laboratory of Zoology Institute, University of Heidelberg, Germany. Mosquito strains were composed of six different strains, namely: *Culex Pipiens Complex*, *Aedes aegypti*, *Aedes vexans*, *Ochlerotatus cantans*, *Ochlerotatus rusticus* and *Anopheles maculipennis*. These were collected from different breeding sites in various localities (Table 1).

**Table 1:** Breeding sites and localities of mosquito strains

| Mosquito Strain | Type of Breeding Site | Locality |
|---|---|---|
| *Cx. pipiens* | stagnant canal | Wittenweier, Germany |
| *Ae. aegypti* | old tire | Cebu City, Philippines |
| *Ae. vexans* | flood plains | Speyer, Germany |
| *Oc. Cantans* | swampy woodlands | Hassloch, Germany |
| *Oc. Rusticus* | swampy woodlands | Hassloch, Germany |
| *An. maculipennis s.l.* | semi-permanent water body | Bobenheim-Roxheim, Germany |

### Collection of mosquito samples

Mosquito samples were collected during larval stage. The collection was done by scooping the mosquito larvae with a fine mesh net. The larvae were placed in a small jar bottles containing water and covered with screens or fine nets.

### Transmission electron microscopy

Individual mosquito samples were placed in a small petri dish with 2.5 % glutaryldehyde in 0.1 M cacodylate buffer (pH 7.4) for the fixation process. Samples were viewed under the stereoscope for sectioning. The abdomens were sectioned into three parts, namely: anterior, middle, and posterior. The most anterior and posterior part of the abdomen were discarded. Each section was placed in a small glass vial which contained 2.5 % glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) for 2 h at 4°C. The samples were washed three times with 0.1 M cacodylate buffer for a period of 10 min. and then washed with 1% osmium tetroxide for 2 h. After osmium tetroxide, the samples were washed 3 times for a period of 30 min. with 0.1 M cacodylate buffer (pH 7.4) and were washed again three times with 0.05 M malic acid buffer (pH 5.2) for a period of 30 min. The samples were transferred to 1% uranyl acetate in 0.05 M malic acid buffer (pH 5.2) for a period of at least 2 h to overnight.

After the samples were soaked in 1% uranyl acetate in 0.05 M malic acid buffer (pH 5.2) (Appendix 3-2), these were washed three times with 0.05 M malic acid buffer (pH 5.2) for a period of 15 min. Tissues were dehydrated three times per step in an increasing concentrations of ethanol, namely: 50, 70, 80, 96, & 100%, each for 10 min [4].

After dehydration, tissues were infiltrated overnight with 1:1 mixture of Spurr pur and 100 % ethyl alcohol. The samples were infiltrated again in a mixture of 3:1 Spurr pur and 100% ethanol for at least 1 h and were further infiltrated with Spurr pur for a period of at least 6 h. Tissues were embedded and polymerized at 60 °C for at least 1 day.

### Trimming and mounting the specimen block for sectioning

The specimen block was firmly clamped into the appropriate specimen holder after removal from the embedding mould for ultra-microtome sectioning. Prior to sectioning, the specimen block was trimmed in order to define the shape and size of the sections. This was done by using a single sided razor blade. Specimen blocks were trimmed in various shapes depending on the form of the mounted specimen.

Glass knives were used in cutting the ultra-thin sections. The glass was cleaned prior to cutting with soap water and dried with a smooth cotton cloth. Upon handling the glass, proper care was taken into consideration in order to avoid finger prints on the narrow sides of the strip where the knife edges were formed. The glass plate was broken into strips or large squares in order to obtain glass knives. The glass knife was blunted as each section was cut and this affected the thickness of the section that was subsequently cut. When the quality of the section deteriorated to less than that was required, a new part of the knife edge was used.

### Semi-thin sections

Knowledge concerning the structure of the specimen as seen in light microscope was very essential before viewing the sections under electron microscopy. Semi-thin sections were very useful in the selection of suitable ultra-thin sections especially if there were several specimens. Additionally, semi-thin sections were important because it showed an evaluation in the preparation technique, such as inadequate penetration of the embedding medium into the denser parts of the specimen. Furthermore, semi-thin sections were done until the region of the ovary of mosquito was observed.

The optimum semi-thin section thickness for light microscopy is between 0.2 and 2 µm. These sections were cut using glass knives in a microtome. The sections were collected carefully from the glass knife without folding the knife edge and placed onto the surface of a glass slide with distilled water. This was done by using a small, fine glass pipette. Excess water in the glass slide was drained off by allowing the slide to dry into the hot plate at 60-80°C. After drying, Richardson's stain was added to the slide that contained the sections. When the stain had dried a little, it was washed immediately with distilled water and dried again on the hot plate. Semi-thin sections were viewed under light microscope.

### Ultra-thin sections

During the start of sectioning, the specimen arm was either operated manually or under automatic control and the knife advanced in small increments of approximately 1 µm between each stroke. Once the contact had been made, the sectioning

was allowed to continue until sufficient sections were obtained or until the sections became already erratic. The ultra-thin sections were collected from the glass knife by using a fine glass pipette.

**Contrasting the ultra-thin sections**

Ultra-thin sections were contrasted two times using uranyl acetate and lead citrate for a period of three min. and were washed with distilled water. Washing was done by dipping the sections 10 times in four small bottles containing distilled water.

## Results

**The ultra-structure of mosquito ovaries without blood meal**

The following transmission electron micrographs presented below were observed based on the microscopic study on the ovaries without blood meal.

Each of the ovaries of mosquito was compose of a reproductive unit called ovarian follicles or epithelial follicles (Fig. 1-1A). There were little cytoplasm that were present in the epithelial cells and the cells were cuboidal with large nuclei (Fig. 1-1A). Beneath the epithelial follicles lay the oocyte having no distinct shape of the nucleus (Fig. 1-1A). Mitochondria are present within the oocyte and several un-oriented microvilli are also observed in the epithelial follicle (Fig. 1- 1B). If the mosquito ovary is unfed with blood, the cells of the ovarian follicles had no continuous epithelium but instead they possess isolated cells with gaps between them. These epithelial follicles were in close proximity and joined by each other at the apical margins by desmosomes (Fig. 1-1 B & C).

The basal surface of the ovarian follicles lay on a basement membrane or basal lamina (Fig.1-2A). The nuclei of ovarian follicles were seen to possess a distinct polymorphic nucleolus which was enveloped by a prominent granular chromosomal mass. Several tracheoles were observed outside the basement membrane of the ovarian follicles (Fig. 1-2B).

Mitochondria were seen very frequently in the ovarian follicles which possess well ordered, parallel arrays of cristae (Fig. 1-3A). Other major inclusion that was seen in the ovarian follicles was the lysosomes (Fig. 1-3B).

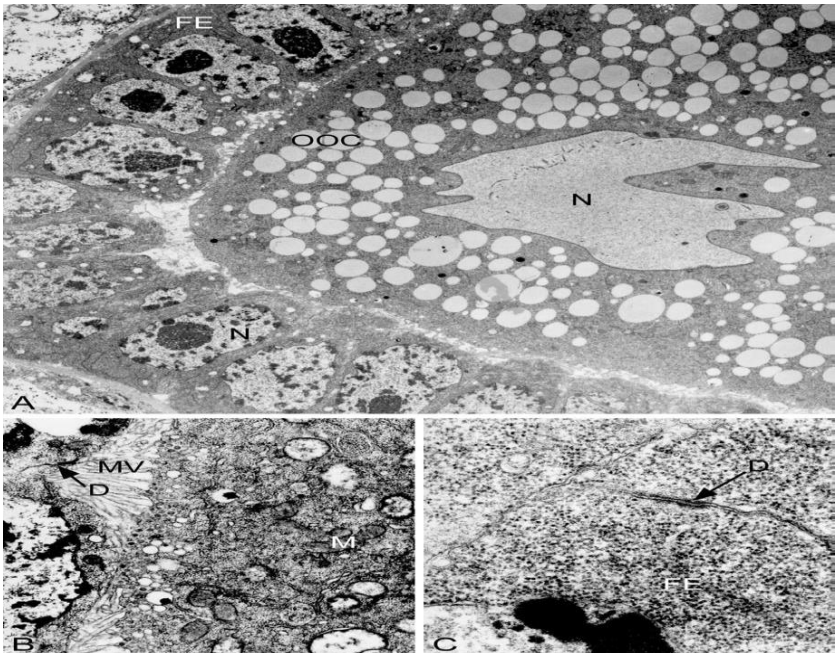


**Fig 1-1A:** Transmission electron micrograph of an epithelial follicles (FE) of *Aedes aegypti* having large nuclei (N). Beneath the epithelial follicles lies the oocyte (OOC) having irregular shape of nucleus (N). X 2 100. B. Mitochondria (M) as well as several un-oriented microvilli (MV) are present in the epithelial follicles. X 14 250. C. Desmosome (D) which joins the epithelial follicles. X 42 000

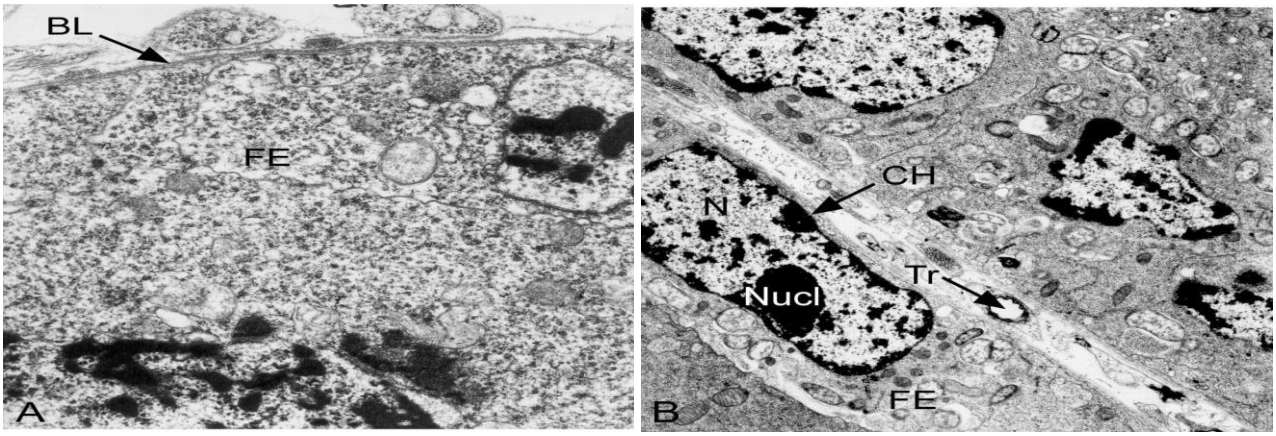


**Fig 1-2A:** Transmission electron micrograph of a basement membrane or basal lamina (BL) in which the basal surface of follicle epithelial cells (FE) are lying in the ovary of *Aedes aegypti*. X 14 250. B. An epithelial follicles of *Aedes aegypti* with nucleus (N) that is enveloped by chromosomal mass (CH) and possess a distinct polymorphic nucleolus (Nucl). Tracheoles (Tr) are present outside the basement membrane of epithelial follicle (FE). X 7 125

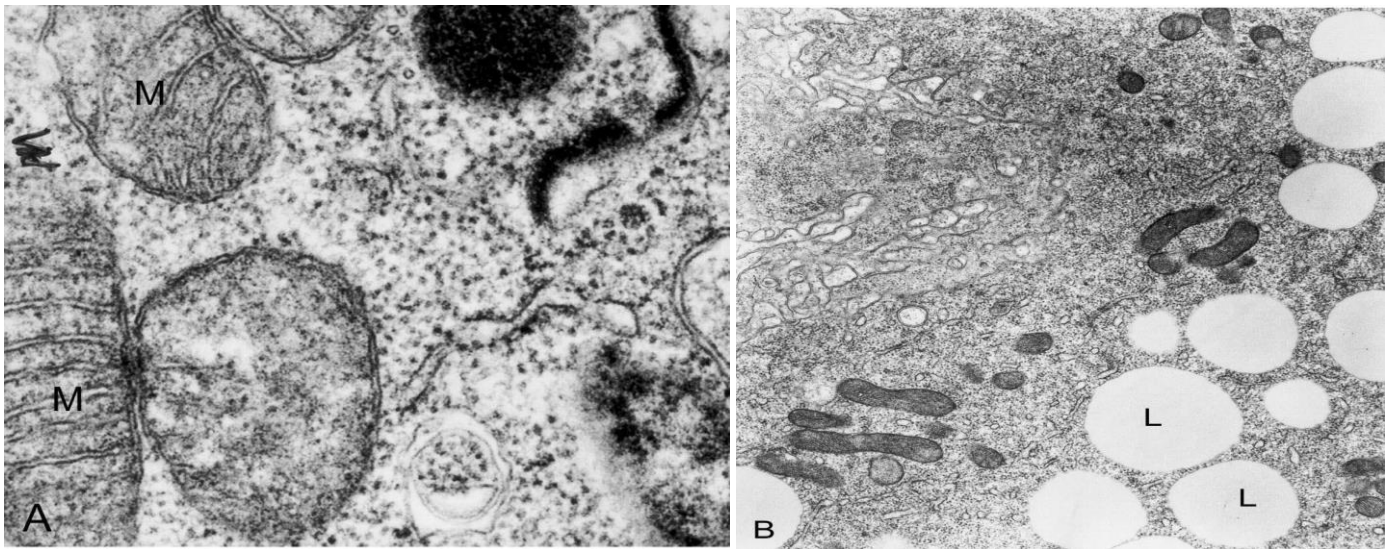


**Fig 1-3A:** Transmission electron micrograph of a mitochondria (M) in an ovarian follicle of *Aedes aegypti*. X 42 000. B. Lysosomes (L) in an ovarian follicle of *Aedes aegypti*. X 14 250

Beneath the epithelial follicles, a single oocyte and a nurse cell were observed (Fig. 1-4A) The nurse cell were relatively large (27μ) with the nuclei alone having the width of three follicular epithelial cells (Fig. 1-4A & B). The granular nucleoplasm had several discrete nucleoli which was limited by a nuclear envelope and by a large number of pores (Fig. 1-4A).

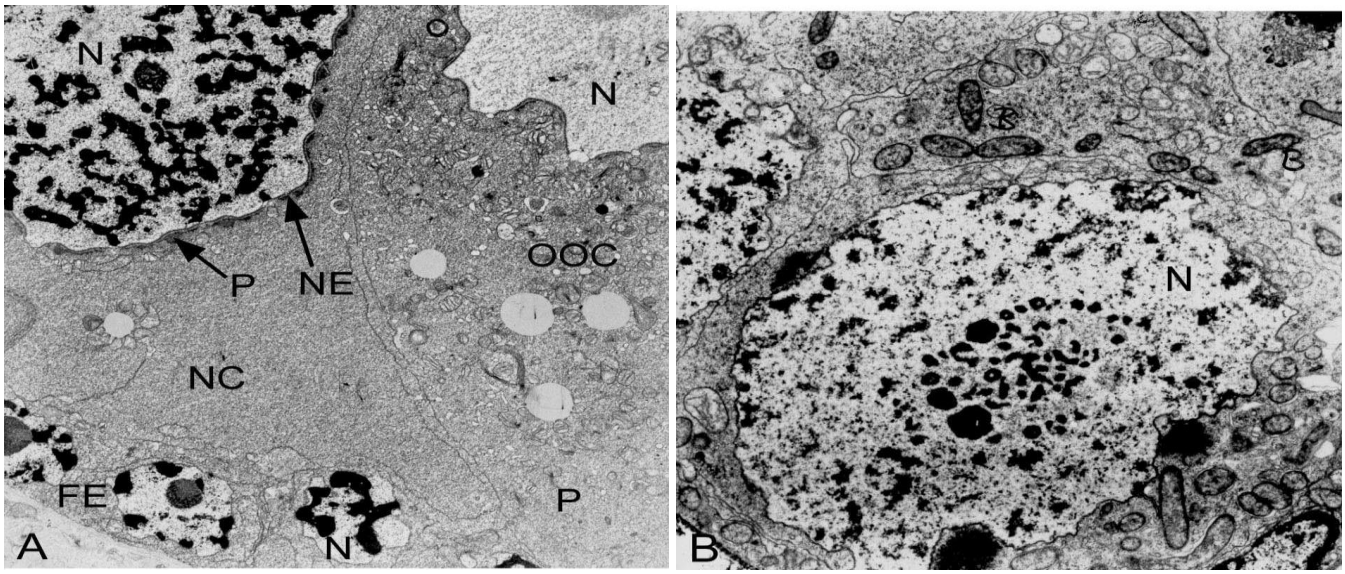


**Fig 1-4A:** Transmission electron micrograph of an oocyte (OOC) and nurse cell (NC) which reside beneath the epithelial follicles (FE) in the ovary of *Aedes aegypti*. **X 3 600**. Nurse cell (NC) showing the large nucleus (N) with nuclear envelope (NE) and nuclear pores (P) in the ovary *Aedes aegypti*. X 7 125. B. Nucleus (N) of a nurse cell in the ovary of *Aedes aegypti*. X 7 125.

The oocyte was identified by its irregularly shaped nucleus (Fig. 1-5A). The nucleolus was a dense, meandering, polymorphic structure which extends long branches out into the nucleoplasm. The chromosomal mass possesses a unique configuration of synaptic chromosomes (Fig. 1-5B). The enveloping nuclear membrane was perforated with pores which provide a distinct separation from the rest of the oocytes (Fig. 1-5B).

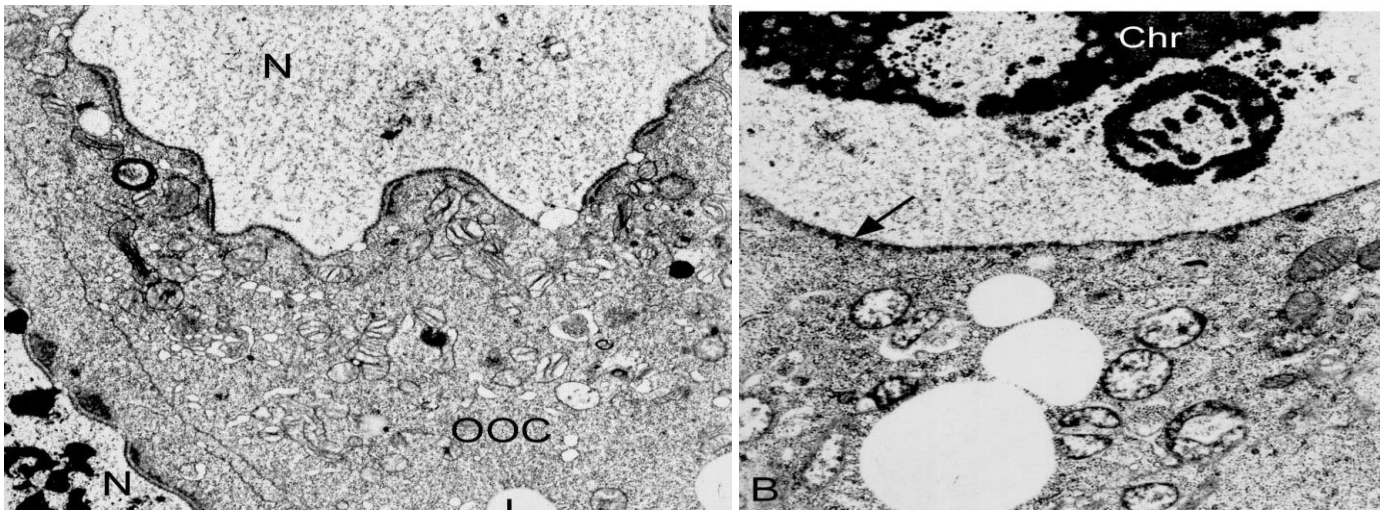


**Fig 1-5A:** Transmission electron micrograph of an oocyte (OOC) with irregular nucleus (N) in the ovary of *Aedes aegypti*. X 14 250. B. Chromosomal mass (Chr) in the nucleus of an oocyte in the ovary of *Aedes aegypti*. X 42 000. Nuclear membrane (arrow) in the oocyte of *Aedes aegypti*. X 42 000

There were several pits or vesicular bodies that were present within the border of microvilli. The vesicular bodies were uniformly small and undeveloped as compared with those observed later when yolk deposition begins after blood meal (Fig. 1-6A). Several lysosomes were also present near to the microvilli (Fig. 1-6B).

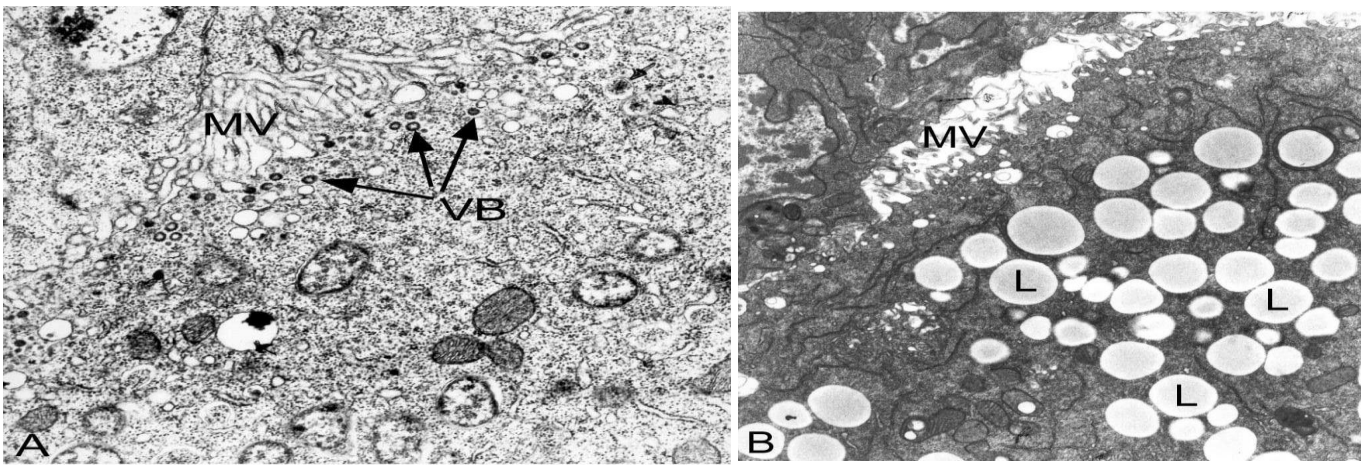


**Fig 1-6A.** Transmission electron micrograph of an oocyte having unoriented microvilli (MV) and vesicular bodies (VB) of *Aedes aegypti*. X 14 250. B. Lysosomes (L) are present near to the microvilli in the oocyte of *Aedes aegypti*. X 7125.

The ultra-structure of an epithelial follicle having no blood meal constitutes the base-line on which the substantial changes that occurred in the oocyte of the blood-fed mosquito. A meal of blood in the mosquito can alter a series of changes in the cell structure that leads to the formation of a mature egg.

**The ultra-structure of mosquito ovaries with blood meal**

After the mosquito had taken a blood meal, some prominent changes occurred in the epithelial follicles. The very prominent changes that occurred were the development of large inter-cellular spaces and a decreased in desmosomal connections which perhaps were associated in the separation of these follicles. The inter-cellular spaces were filled up with finely particulate and somewhat flocculent in appearance (Fig. 1-7A). The distribution of this material throughout the inter-cellular spaces was essentially uniform.

Several numbers of microvilli (also evident during the resting stage but undeveloped) covered the area of the oocyte facing the epithelial follicles of which during seven h after blood meal, push towards the extra-cellular spaces created by the separation of the epithelial follicle cells (Fig. 1-7B). The microvilli vary in length and were not regularly distributed like those of the typical intestinal epithelium. It seemed, however, that they had a uniform diameter (60μm) especially in their greatest extension from the surface (Fig. 1-7B).

Several small protein bodies were observed after the mosquito had taken a blood meal (Fig. 1-8A). A unit membrane was seen which later fused to form a yolk (Fig. 1-8B). Through the fusion of various units the larger protein bodies of the mature oocyte developed (Fig. 1-8C).

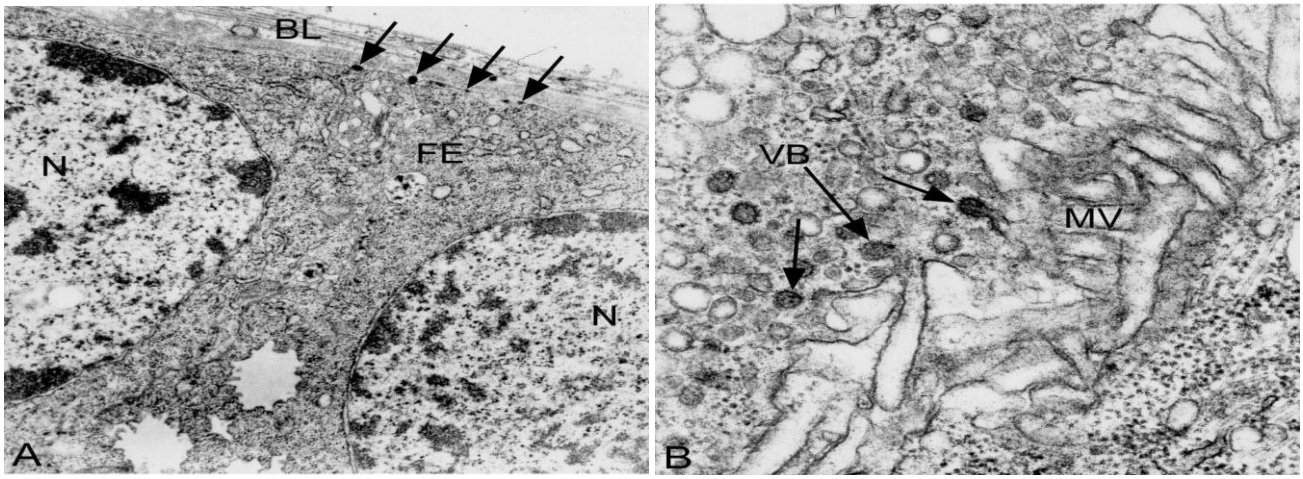


**Fig. 1-7A**. Transmission electron micrograph of inter-cellular spaces (arrows) in the epithelial follicles (FE) of *Aedes aegypti*. X 14 250. B. Microvilli (MV) and the vesicular bodies (VB) in the ovary of *Aedes aegypti*. X 14 250

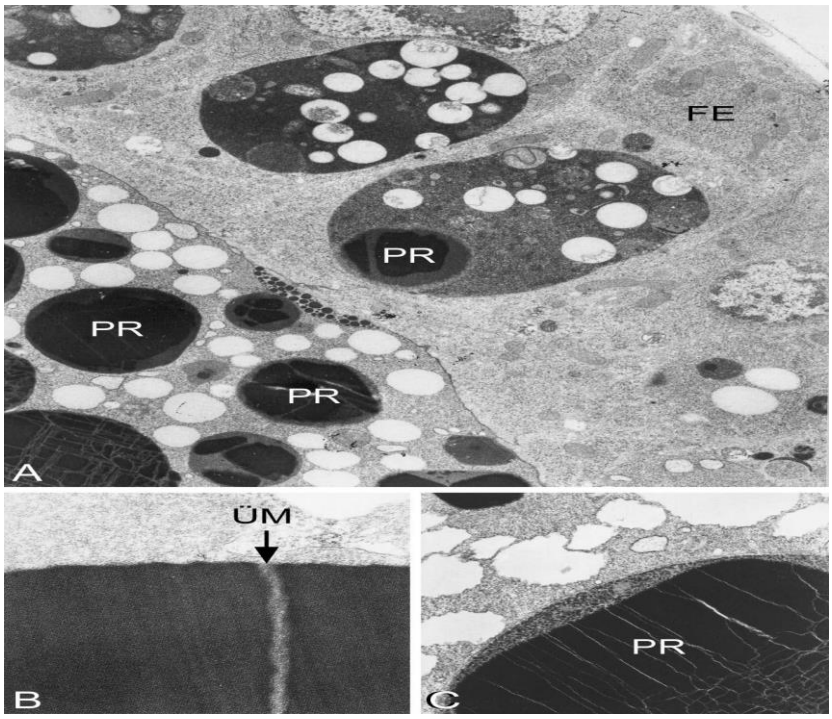


**Fig. 1-8A.** Transmission electron micrograph of protein bodies (PR) in a mature oocyte of *Aedes aegypti*. X 42 000. B. Unit membrane which later fuses to form a yolk (UM). 42 000. C. Yolk granules (PR) in the ovary of *Aedes aegypti*. X 14 250.

**Discussion**

A meal of blood or controlled diet leads to a series of changes in cell structures in the reproductive organ of female mosquitoes that quickly results to the formation of matured eggs. One of the prominent changes observed in the ovary of mosquitoes having blood meal is the presence of various un-oriented microvilli in the area of the oocyte which is adjacent to the epithelial follicles (Fig. 1-7B). These microvilli can also be observed in ovary of mosquitoes having no blood meal but they are smaller in size. Roth and Porter (1964) observed the same structural changes and they cited that the great numbers of microvilli are push into the extracellular spaces after 7 h of a blood meal. As it is depicted in this study, these microvilli are somewhat not uniform in length and are not regularly dispose as in typical intestinal epithelium which was described by Brandt [7].

Other prominent structural changes are observed in the region of the oocyte and epithelial follicles of the ovary. These changes are the occurrence of large intercellular spaces and the decrease in desmosomal connections in the epithelial follicle cells (Figs. 1-7A & 1-1B & C). The epithelial follicle cells open channels between the extra-epithelial follicle space and the surface of the oocyte for the exchange of materials for the development of yolk [4].

Furthermore, pits or vesicular bodies are developed after a mosquito has taken a blood meal (Fig. 1-6A). Pits of similar structure obtained from this study have been reported in many cell types [8-10]. They are not to be confused with the simpler pits found especially in smooth muscle cells and blood vascular endothelial cells. The latter have simple, clean limiting membranes and are smaller in diameter [11].

The observations of pits in this study are in agreement with some other authors.[1,4] These authors interpreted that these pits are engaged in pinocytocis which is associated in the uptake of materials for yolk formation. In the study done by Roth and Porter (1964), it was found that a number of pits approximately 300,000 are observed in the oocyte after 7 h of a blood meal. This result in a 15 times increase of the number of pits found in the oocyte after the mosquito has taken a blood meal. Pits or vesicular bodies fuse to form small

crystalline yolk droplets which subsequently coalesce to form large proteid bodies (Roth & Porter, 1964). Apparently, through the fusion of these various units of proteid bodies, the mature oocyte developed as depicted in this study in Figure 1-8A, B and C. The implication from the above observations and the literature on yolk synthesis is that these pits are responsible for taking up materials from the extracellular space of the follicle and contribute it to yolk granule formation [12- 16].

There are reasons to believe that yolk deposition in the developing oocyte of a mosquito is accomplished by the removal of the protein from the blood [3]. All of the structural mechanisms associated with rapid synthesis of proteins or lipoproteins, especially for segregation and storage in granules, appear in the fine structure of the epithelial follicles. Indeed, the very un-usual structural feature does appear, which is seemingly involved in the yolk deposition, are the development of pits or vesicular bodies [1]. Generally, yolk develops rapidly, in fact, synthesis and storage are essentially completed at least 25 hour after blood meal [4, 17]. By 4 hour after the blood meal, these changes in the cell structure are already in evidence, and after 7 hour, they are very obvious [4].

**Acknowledgements**

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