

Culex quinquefasciatus vitellogenesis: morphological and biochemical aspects

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The vitellogenic process in Culex quinquefasciatus, which is triggered by a blood meal, involves the synthesis, distribution and storage of the nutrients necessary for embryo development. The fat body of an adult female Cx. quinquefasciatus revealed two cell types: large trophocytes and small, eosinophilic, "oocyte-like" cells, which show no morphological changes throughout the gonotrophic cycle. Trophocytes, which only begin to synthesise vitellogenin (Vg) 12 h post-blood meal (PBM), undergo a series of morphological changes following engorgement. These changes include the expansion of the rough endoplasmic reticulum (RER) and Golgi complex, which are later destroyed by autophagosomes. At 84 h PBM, trophocytes return to their pre-engorgement morphology. The ovarian follicles of non-blood-fed Cx. quinquefasciatus contain a cluster of eight undifferentiated cells surrounded by follicular epithelium. After engorgement, the oocyte membrane facing the perioocytic space increases its absorptive surface by microvilli development; large amounts of Vg and lipids are stored between 24 and 48 h PBM. Along with yolk storage in the oocyte, follicular cells exhibit the development of RER cisternae and electron-dense granules begin to fill the perioocytic space, possibly giving rise to endochorion. Later in the gonotrophic cycle, electron-dense vesicles, which are possible exochorion precursors, fuse at the apical membrane of follicular cells. This fusion is followed by follicular cell degeneration.

Key words: *Culex quinquefasciatus* - vitellogenesis - immunolabelling - morphology - fat body - ovary

During the course of oogenesis, insects accumulate large amounts of resources inside their eggs in the form of proteins, lipids, carbohydrates and other minor components. These supplies or "yolk" are critical for embryonic development and are produced in both autotrophic and heterotrophic manners (Tadkowski & Jones 1978, de Bianchi et al. 1985, Raikhel & Dhadialla 1992, Attardo et al. 2005). Yolk formation in mosquitoes has been extensively studied in *Aedes aegypti* (Raikhel 1984, 1987, Dhadialla & Raikhel 1990, Snigirevskaya et al. 1997), a species that has become the model for mosquito oogenesis.

The insect fat body is a multifunctional organ involved in intermediary metabolism and the storage of fat, glycogen and protein (Chapman 1998). Trophocytes are the main cell type of fat body tissue. Their cytoplasm is mainly filled with lipid droplets, while the remaining space is occupied by the nucleus and a thin perinuclear strip containing a few organelles such as rough endoplasmic reticulum (RER), Golgi complex, mitochondria and lysosomes.

The sequence of ultrastructural changes occurring in the trophocytes of vitellogenic *Ae. aegypti*, includes the prominent expansion of the RER and Golgi complex, and

an increase in the number of mitochondria (Raikhel & Lea 1983). In *Ae. aegypti*, vitellogenin (Vg), the most abundant yolk protein precursor, is synthesised exclusively in fat body cells (Hagedorn & Judson 1972, Hagedorn et al. 1973), secreted into the haemolymph and internalised by receptor-mediated endocytosis by developing oocytes (Snigirevskaya et al. 1997, Sappington & Raikhel 1998). At the end of vitellogenesis, fat body cells are characterised by activation of the lysosomal machinery with the formation of numerous autophagosomes (Raikhel & Lea 1983, Raikhel 1986, Snigirevskaya et al. 1997).

Mosquito ovaries are dorsolaterally situated in the posterior region of the abdomen and each is composed of functional units called ovarioles (Clements 1992). Each ovariole contains a germarium, where germ cells proliferate and follicles are formed, and a vitellarium, where oocytes develop and undergo the storage of yolk proteins. During each gonotrophic cycle, the follicles within each ovariole mature synchronously. Each follicle is surrounded by a single layer of follicular cells, which are derived from somatic tissue and will secrete components of the eggshell (Clements 1992, Raikhel & Dhadialla 1992).

Culex quinquefasciatus is a cosmopolitan mosquito that is highly anthropophilic and completely adapted to urban conditions with a larval stage capable of developing in highly polluted water collections. This buzzing and biting mosquito is responsible for great nocturnal discomfort and allergic responses (Malafronte et al. 2003). Additionally, *Cx. quinquefasciatus* transmits pathogens such as *Wuchereria bancrofti*, one of the agents of lymphatic filariasis and is a competent vector of neurotropic viruses (Gaunt et al. 2001). The St. Louis

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and Japanese encephalitis viruses, eastern and western equine encephalomyelitis viruses, Rift Valley virus and West Nile virus are efficiently transmitted by *Cx. quinquefasciatus* (Vaidyanathan & Scott 2007, Hamer et al. 2008). Therefore, it is important to control the populations of this mosquito and the transmission of its associated pathogens.

Although vector mosquitoes of the *Aedes*, *Anopheles* and *Culex* genera share many developmental, biochemical, behavioural and morphological traits, significant differences do occur. For example, *Cx. quinquefasciatus* diverges from mosquitoes of other genera, i.e., *Aedes* and *Anopheles*, in the morphology of its salivary glands and saliva composition (da Cunha Sais et al. 2003, Ribeiro et al. 2004) in the cellular and biochemical processes involved with blood digestion and haem detoxification (Okuda et al. 2002, 2007) and in its response to odorants and biting behaviour (Van Essen et al. 1994). Therefore, comparative studies may provide a basis for the development of novel strategies and the improvement of the current approaches for vector control.

In this paper, we describe the changes in the fat body and ovaries of *Cx. quinquefasciatus* during the first gonotrophic cycle.

MATERIALS AND METHODS

Animals - *Cx. quinquefasciatus* (PIN strain) mosquitoes were raised at 26°C in 70-80% relative humidity and a photoperiod of 12 h dark-12 h light. Adults were fed a 10% sucrose solution *ad libitum*. When necessary, 5-7-day-old adult females were fed on Balb/c mice anaesthetised with 0.3 mg/kg of xylazine hydrochloride (Calmium, Agner União) plus 30 µg/kg of acepromazine (Acepran, Univet SA, São Paulo, Brazil).

Gel electrophoresis - Ovaries and fat bodies (i.e., abdomens free of gut, Malpighian tubules and ovaries) (Fallon et al. 1974) were dissected before engorgement and at 3, 6, 9, 12, 24, 48, 72 or 96 h post-blood meal (PBM). They were then homogenised in PBS containing 1 µl/mL of a cocktail of protease inhibitors (50 µg/mL leupeptin, 5 µg/mL pepstatin, 5 µg/mL chymostatin, 5 µg/mL antipain, 5 µg/mL PMSF) and 5 µM E-64; the protein content was determined using the Bradford assay (Bradford 1976). SDS-PAGE was performed on 8% polyacrylamide gels (Laemmli 1970) and proteins were visualised by staining with Coomassie Brilliant Blue R-250. Molecular masses were estimated using protein standards (Bio-Rad Laboratories).

Antibody production - Monospecific polyclonal antisera against yolk protein were raised in Balb/c mice. *Cx. quinquefasciatus* ovaries (48 h PBM) were subjected to SDS-PAGE. After electrophoresis, the Coomassie blue-stained bands corresponding to the 86 and 192 kDa vitellin fractions were excised from the gel and minced the products were used independently for two intraperitoneal immunisations with an interval of 15 days between them. Seven days after the second immunisation, the animals were bled to obtain the sera.

Immunoblotting - Proteins subjected to SDS-PAGE were electroblotted onto a nitrocellulose membrane. The membrane was blocked overnight with Tris-buffered saline [TBS; 0.02 M Tris(hydroxymethyl)aminomethane, 0.15 M NaCl, pH 8.2] pH 7.4 containing 5% non-fat milk (blocking buffer). Membrane strips were incubated with a 1:600 dilution of both anti-vitellin sera (anti-Vg, small fraction; anti-VG, larger fraction) in blocking buffer for 90 min with agitation. Mouse pre-immune serum was used as a control. After three serial washes (5 min each) in TBS pH 7.4, the bound antibodies were detected with peroxidase-conjugated goat anti-mouse polyvalent immunoglobulins (Cappel) diluted 1:1,500 in blocking buffer. After another set of washes, the immunoblots were developed by the addition of H₂O₂ and 4-Chloro-1-Naphthol. The reactions were stopped by transferring the membrane to deionised water. Fig. 1 shows the specificity of the antisera with each recognising a single band of the expected mass (86 and 192 kDa).

Morphology - Five insects were used for each analysis, which was performed before engorgement and at 6, 12, 18, 24, 36, 48, 60, 72, 84 and 96 h PBM. Fat bodies and ovaries were dissected in PBS under a stereoscopic microscope.

Fat bodies were fixed for 2 h at 4°C in 3.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2. Following post-fixation with 1% osmium tetroxide, the samples were dehydrated in a graded ethanol series.

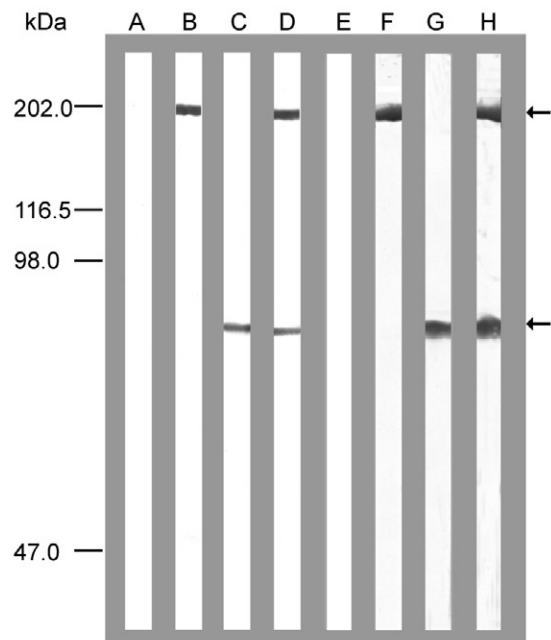


Fig. 1: immunoblot of fat body (A-D) and ovary (E-H) extracts. Both antibodies are highly specific each recognizing a single band. Arrows indicate the large (192 kDa) and small (86 kDa) polypeptides of vitellogenin (Vg). A: pre-immune serum; B: anti-VG; C: anti-Vg; D: anti-VG plus anti-Vg; E: pre-immune serum; F: anti-VG; G: anti-Vg; H: anti-VG plus anti-Vg.

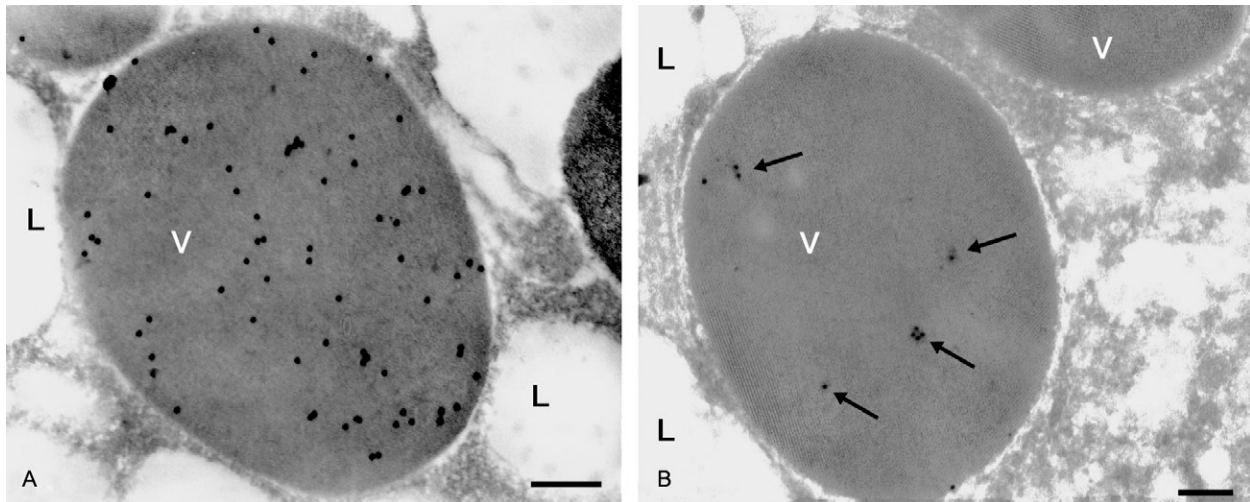


Fig. 2: immunocytochemistry of ovaries of 24 h post-blood meal *Culex quinquefasciatus* using the polyclonal antibodies anti-vg (A) and anti-VG (B, arrows). Note the high affinity of each antibody and the low sensibility of anti-VG in this system. L: lipid droplets; V: vitellin granules. Bar: 200 nm.

Ovaries were fixed for 2 h in 0.5% glutaraldehyde and 4% paraformaldehyde in 0.1 M sodium cacodylate buffer pH 7.2 at RT. After post-fixation with 1% osmium tetroxide, samples were contrasted *en bloc* with 2% uranyl acetate in 70% acetone and dehydrated in a graded acetone series. After dehydration, the samples were embedded in Spurr's resin. Semithin sections (200-300 nm) were stained with toluidine blue for light microscopy studies. Ultrathin sections (70-80 nm) were stained with uranyl acetate and lead citrate and studied at 80 kV in a JEOL 100CX transmission electron microscope.

Immunocytochemistry - Fragments of *Cx. quinquefasciatus* ovaries 48 h PBM were fixed for 1 h in 0.5% glutaraldehyde, 4% paraformaldehyde and 0.2% picric acid in 0.1 M cacodylate buffer (pH 7.2) containing 1 mM CaCl_2 . After fixation, the fragments were embedded in LR White and placed in gelatin capsules for polymerisation at 37°C. Silver sections placed onto nickel grids were washed in TBS and incubated as described by Okuda et al. (1999). Fig. 2A, B illustrates the specific labelling of the vitellin granules. Due to the higher sensitivity of the anti-vg antibody in immunocytochemistry, this antibody was also chosen for the immunoblot experiments.

Ethics - All procedures involving mice manipulation were approved by the Ethical Committee on Animal Experiments of the Institute of Biomedical Sciences of São Paulo University.

RESULTS

The fat body - As described in other insects, the fat body of *Cx. quinquefasciatus* is organised into a sheet of cells distributed beneath the body wall, suspended in the haemolymph and involving the viscera (Fig. 3A).

Before engorgement, light microscopy of the *Cx. quinquefasciatus* fat body revealed two cell types. Most

of the organ is composed of large trophocytes with a highly vacuolated cytoplasm. Mingled with them are small cells characterised by their eosinophilic cytoplasm (oenocyte-like cells) and occurring in isolation or in small groups (Fig. 3A). At the ultrastructural level, oenocyte-like cells display a dense cytoplasm containing numerous mitochondria with noticeable cristae and electron-dense granules; the nuclei show a prominent nucleolus and abundant peripheral heterochromatin (Fig. 3B). Oenocyte-like cells showed no ultrastructural changes throughout the first gonotrophic cycle. These cells not appear to be involved in the changes in the protein profile observed in Fig. 4A, C.

The trophocytes of non-blood-fed females are large cells with a cytoplasm that is almost entirely occupied by conspicuous lipid droplets and some glycogen depots. A thin ring of poorly developed cytoplasm surrounds a spherical nucleus with a prominent nucleolus and dispersed heterochromatin (Fig. 5A). Scarce, small cisternae of the RER are found at 6 h PBM, usually in the vicinity of the nucleus (Fig. 5A, insert). At 12 h PBM, they are already easily noticeable and some of them are spiralled. RER cisternae continue to increase in number and, together with Golgi cisternae, nearly fill the perinuclear cytoplasm at 24 h PBM (Fig. 5B). At this point, the electron-dense vesicles and granules emerging from the Golgi complex are also evident (Fig. 5C). Only 12 h after a blood meal, the presence of Vg is noticeable in the fat body extracts (Fig. 4A, C), which is consistent with the scarce development of RER observed at the previous times (Fig. 5A, insert). Vg remains detectable until 48 h PBM. At that time, the biosynthetic organelles begin to decline and autophagosomes are observed between 48-72 h PBM (Fig. 5D, E). Only at 84 h PBM do the trophocytes return to the appearance that they had before the blood meal (Fig. 5F).

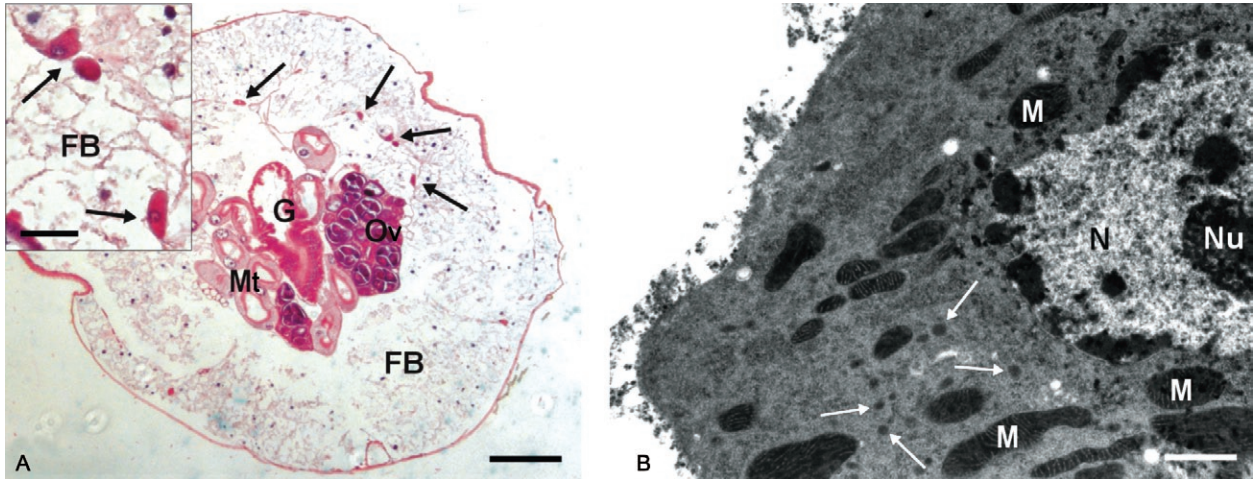


Fig. 3: *Culex quinquefasciatus* 7-days-old non blood-fed female. A: light micrograph of a haematoxylin-eosin stained cross-section through mosquito abdomen. Ovaries (Ov), midgut (G) and Malpighian tubules (Mt) are surrounded by fat body cells (FB), which fill all the space beneath the cuticle. Arrows point oenocyte-like cells, enlarged in the insert. Bar: 50 μ m, insert: 10 μ m; B: electron micrograph of an oenocyte-like cell with characteristic electron-dense cytoplasm containing abundant mitochondria (M) and tiny electron-dense granules (arrows). N: nucleus; Nu: nucleolus. Bar: 1 μ m.

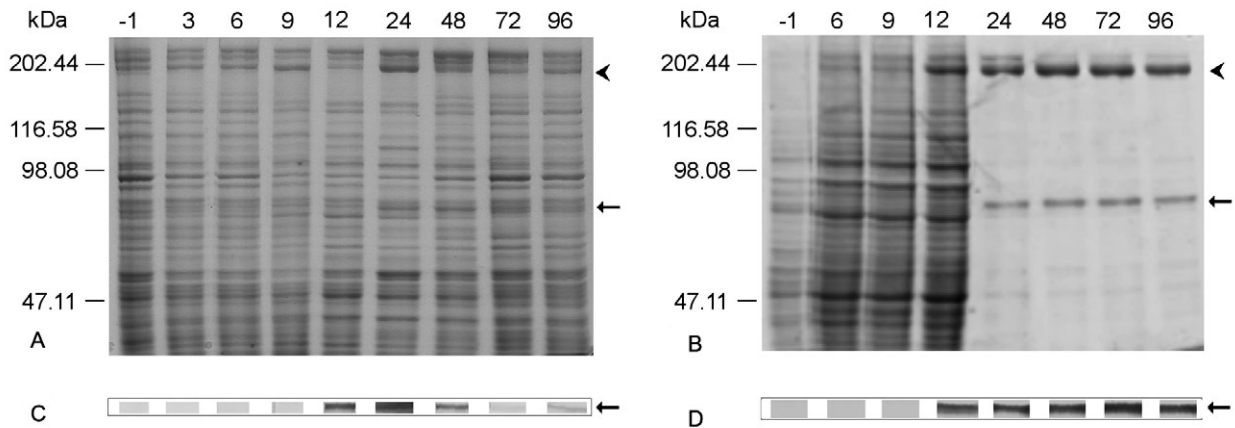


Fig. 4: protein profile analysis of fat bodies (A) and ovaries (B) of *Culex quinquefasciatus* after blood meal. Vitellogenin (Vg) olypeptides of around 192 (arrowheads) and 86 (arrows) kDa were observed only after blood meal. The numbers above lanes indicate hours post blood meal. Immunoblot (anti-vg, arrows) of fat bodies (C) and ovaries (D) of *Cx. quinquefasciatus*. kDa: molecular weight.

The ovaries - Ultrastructure of pre-engorged *Cx. quinquefasciatus* ovaries reveals follicles formed by a set of undifferentiated cells, several of them exhibiting prominent nucleolus. This set of cells is surrounded by a monolayer of flattened cells with dense cytoplasm, the follicular epithelium (Fig. 6A), which does not display any morphological sign of synthetic activity (endoplasmic reticulum and/or Golgi cisternae).

Only after engorgement can the oocyte be distinguished from the remaining cells that give rise to the nurse cells. Soon after a blood meal, the oocytes develop microvilli on their plasma membranes (Fig. 6B, C), which border on the

perioocytic space (between the follicular cells and the oocyte). Coated vesicles are noticeable in the peripheral cytoplasm (Fig. 6B, insert). The accumulation of Vg in the ovary extracts starts to be noticeable around 12 h PBM (Fig. 4B, D). Concomitantly, the formation of vitellin granules and the accumulation of lipid droplets are observed (Fig. 6B). During the subsequent developmental stages, *Cx. quinquefasciatus* oocytes undergo few morphological changes besides their obvious growth. The storage of vitellin and lipids continues and at 24 h PBM the yolk granules occupy almost the entire cytoplasm (Fig. 6C, insert). Only at 96 h PBM does glycogen begin to accumulate (Fig. 6F).

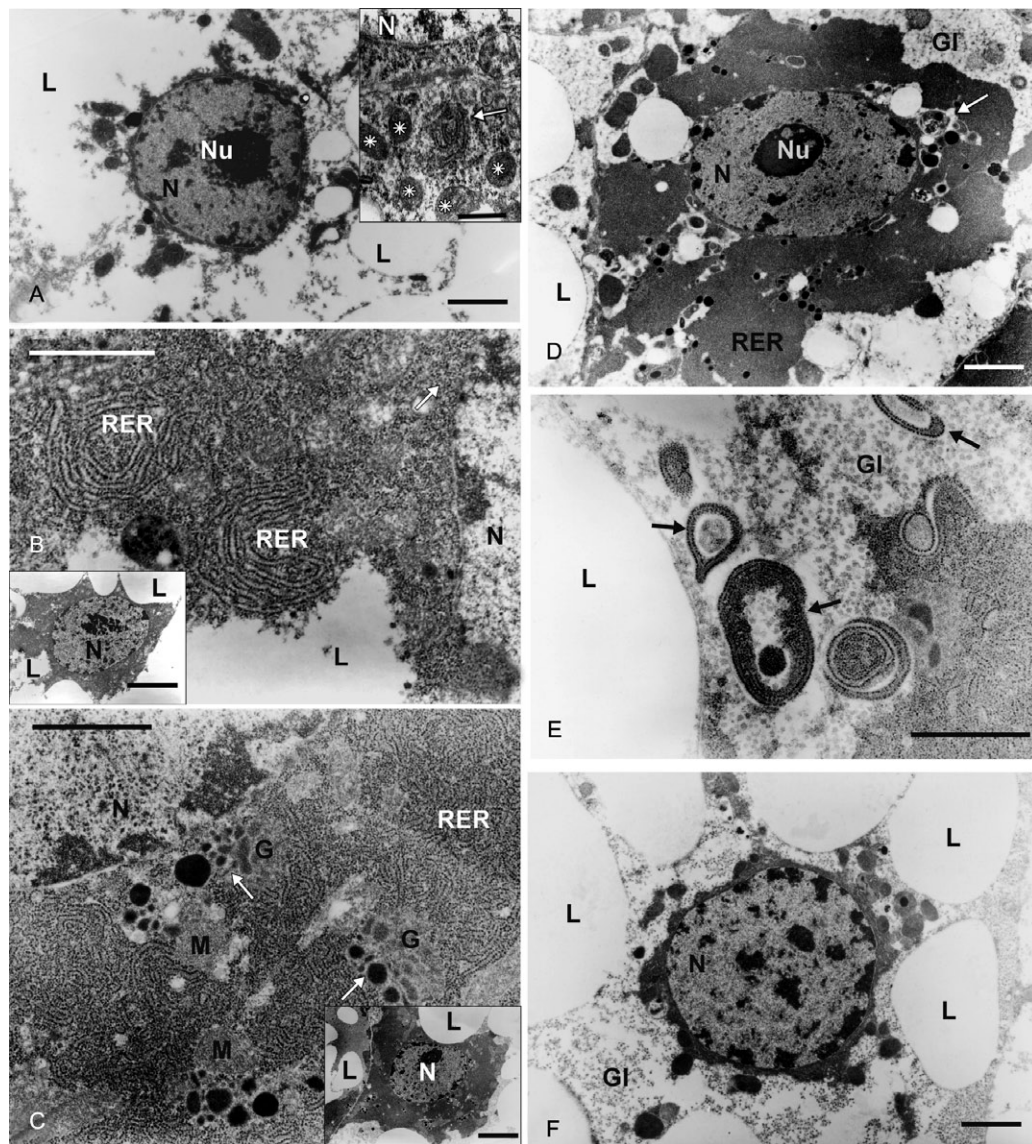


Fig. 5: electron micrographs of fat body of *Culex quinquefasciatus*. A: typical trophocyte of a 7-days-old non blood-fed female with its cytoplasm almost entirely occupied by lipid droplets (L). Nu: nucleolus (Bar: 2 μ m). The insert shows a 6 h post-blood meal (PBM) trophocyte. Small rough endoplasmic reticulum (RER) cisterna of circular profile (arrow) is seen at the nucleus (N) neighbouring. Asterisks mean mitochondria (M). Bar: 500 nm; B: 12 h PBM trophocyte. Observe the circular profiles of the cisternae (RER). Bar: 500 nm. The insert shows the already developed cytoplasm around the N. Bar: 4 μ m; C: 24 h PBM trophocyte. Insert shows the well developed perinuclear cytoplasm that, besides to numerous RER cisternae, exhibits transport vesicles (arrows) in association with G. Bar: 1 μ m. Insert: 2 μ m; D: 48 h PBM trophocyte. Perinuclear cytoplasm begins to present autophagosomes (arrow). Gl: glycogen. Bar: 2 μ m; E: 48 h PBM trophocyte. Presence of numerous autophagosomes (arrows) indicating the degradation of the synthetic machinery. Bar: 1 μ m; F: 84 h PBM trophocyte. The perinuclear cytoplasm has returned to the same characteristics of non-blood fed mosquito. Bar: 2 μ m.

The follicular cells begin to display RER cisternae at approximately 24 h PBM when small electron-dense granules are also noticeable in the perioocytic space (Fig. 6C). At 48 h PBM, RER cisternae occupy most of the cytoplasm (Fig. 6D), while electron-dense plaques that increase in number and size distort the surface microvilli of the oocyte (Fig. 6D). Simultaneously, small electron-dense vesicles can be observed in the apical region of the follicular cells (Fig. 6D, insert). These vesicles seem to merge, forming

large dense plaques at the inner side of the follicular cell membrane at 60 h PBM (Fig. 6E). At this time, the follicular cell cytoplasm already contains several vacuoles (Fig. 6E). Degeneration of the follicular cells takes place during a process that occurs from 60 h PBM till approximately 84 h PBM, when the cells have completely degenerated (Fig. 6F). Oviposition occurs at approximately 90 h PBM (87.39 ± 5.46 , according to our observations) and concludes the gonotrophic cycle.

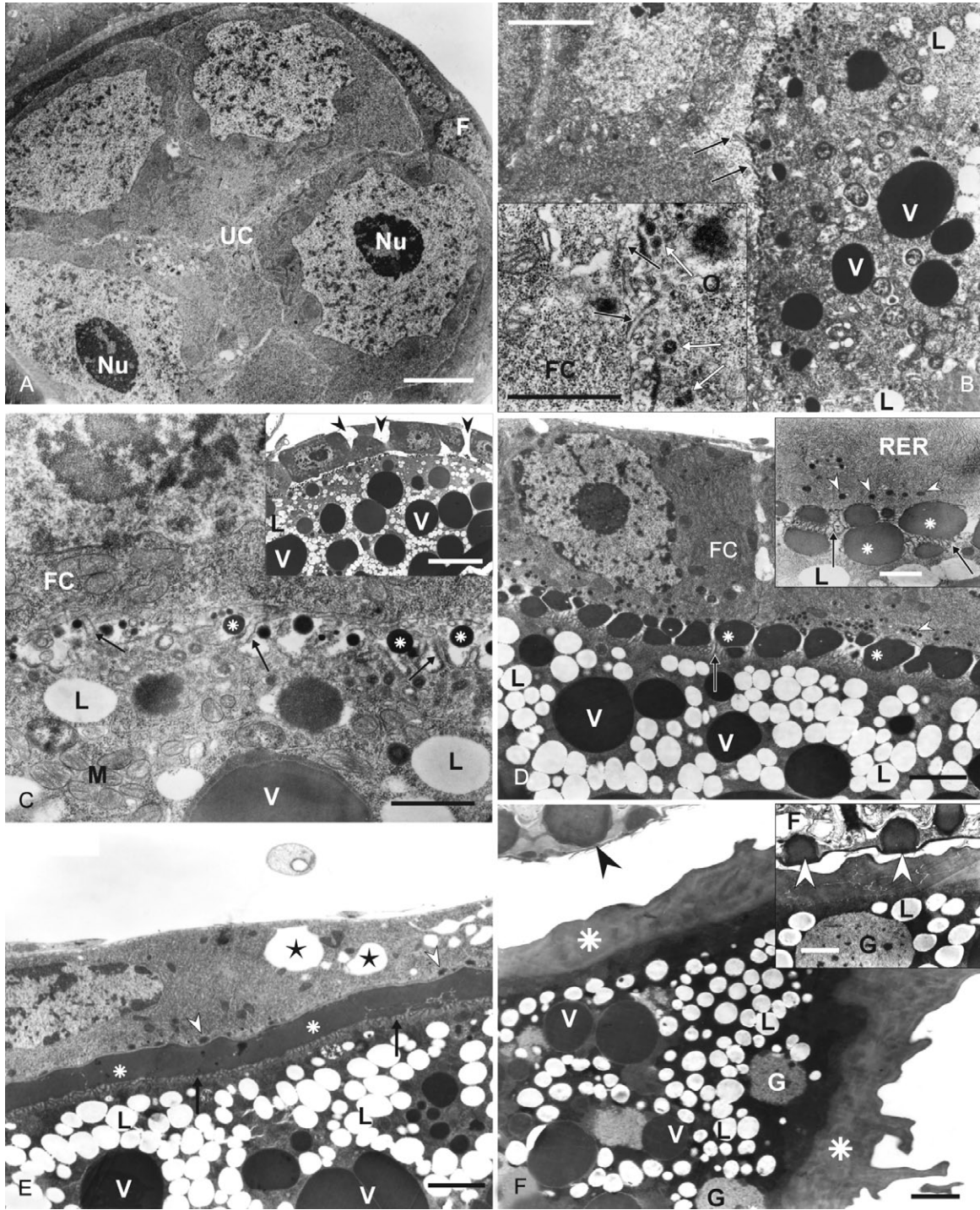


Fig. 6: electron micrographs of *Culex quinquefasciatus* ovarian follicle. A: before the blood meal, the primary follicle is constituted by undifferentiated cells (UC) surrounded by follicular epithelium (F). Nu: nucleolus. Bar: 3 μ m; B: 12 h post-blood meal (PBM) follicle. Oocyte cytoplasm already presents vitellin granules (V) and lipid droplets (L). Microvilli (arrows) of oocyte membrane facilitate the nutrients uptake, evidenced by abundant vesicles in the oocyte apical cytoplasm (white arrows, insert). FC: follicular cell. Bar: 2 μ m, insert: 1 μ m. C: 24 h PBM follicle. Insert illustrate the great quantity of mitochondria (M), L and V inside the oocyte as well as the broad channels (arrowhead) in between the FCs. Electrondense granules (asterisks), putative endochorion precursors, fill the perioocytic space between microvilli (arrows). Bar: 1 μ m, insert: 8 μ m. D: 48 h PBM follicle. Well developed rough endoplasmic reticulum (RER) occupies almost the entire FC cytoplasm, while electrondense vesicles (arrowheads) are noticeable in the apical region. Perioocytic space is filled with choriionic plates (asterisks) that distort the microvilli (arrows) architecture. Bar: 2 μ m, insert: 1 μ m; E: 60 h PBM follicle. Fcs present several intracellular vacuoles (stars) suggesting the beginning of cell degeneration, however dense vesicles (arrowheads) are still noticeable in the apical region. Endochorion (asterisks) is almost completely formed squashing the oocyte microvilli (arrows). Bar: 2 μ m; F: 84 h PBM follicle. Besides L and V, oocyte now exhibits glycogen depots (G). Endochorion (asterisks) is now a continuous electrondense line. Electrondense plaques (arrowheads) suggestive of exochorion, protrude onto the apical membrane of the FC, totally degenerated. Bar: 2 μ m, insert: 1 μ m.

DISCUSSION

As early as 1978, Atlas et al. suggested that the *Culex pipiens fatigans* yolk protein has a native molecular mass of 380 kDa, contains protein-bound phosphate, lipid and carbohydrate and is composed of two polypeptides of 80 and 160 kDa. Insect Vgs are synthesised as precursors that undergo processing as well as proteolytic cleavage into two or more subunits of smaller size (Dhadialla & Raikhel 1990). *Ae. aegypti* Vg is also formed by two subunits of 200 and 65 kDa (Raikhel 1987, Bose & Raikhel 1988, Dhadialla & Raikhel 1991). The difference in mass between the two mosquito Vgs is probably due to post-translational modifications such as glycosylation (Bose & Raikhel 1988). SDS-PAGE of fat body and haemolymph of *Cx. quinquefasciatus* only allow to visualise the larger polypeptide of Vg (possibly the smaller is masked by the smaller polypeptide of lipophorin because both have nearly the same mass (AF Cardoso, unpublished observations). The molecular mass of the Vg polypeptides is consistent with the expected sizes listed in the annotation of the genome (XP_001857970.1). The amino acid sequence has a predicted mass of 241.609 kDa, while our studies indicate that *Cx. quinquefasciatus* Vg has a molecular mass of 278 kDa. The difference is probably due to the posttranslational modifications that occur in the trophocyte. Trials using concanavalin-A confirmed that *Cx. quinquefasciatus* Vg is glycosylated (AF Cardoso, unpublished observations).

When analysing the similarities and differences between the oogenesis processes in *Cx. quinquefasciatus* and *Ae. aegypti*, the first thing that catches one's attention is the length of oogenesis and the oviposition time. In simultaneous experiments conducted under the same environmental conditions, *Ae. aegypti* females lay eggs sooner after a blood meal (63 ± 1 h PBM) (F de Almeida, unpublished observations) than *Cx. quinquefasciatus* females (87.39 ± 5.46 h PBM). The oviposition time that we determined for *Ae. aegypti* is in agreement with that communicated by Gomes et al. (2006). Morphological observations of the *Cx. quinquefasciatus* fat bodies and ovaries prior to and during oogenesis were compared to those previously described for the better-studied *Ae. aegypti*.

In a general, the appearance of the fat body in female *Cx. quinquefasciatus* mosquitoes is similar to that described for other mosquitoes. Four cell types have been described in arthropod fat bodies. Trophocytes are involved in intermediate metabolism and storage and the synthesis of Vg. Urocytes are engaged in the storage and secretion of uric acid. Mycetocytes are associated with the fat body in some insect species that have intracellular symbiotic microorganisms (Keeley 1978). Oenocytes are associated with the synthesis of hydrocarbons, precursors of cuticle lipids and pheromones in cockroaches (Fan et al. 2003).

The *Cx. quinquefasciatus* fat body is mainly composed of large trophocytes, but we also observed oenocyte-like cells. Unlike the description of *Ae. aegypti* oenocytes presented by Tadkowski et al. (1977), we failed to observe any change in these cells throughout the entire gonotrophic cycle.

When compared with other descriptions, trophocytes of the *Cx. quinquefasciatus* fat body exhibit what appears to be a temporal discrepancy. A previtellogenic phase, in which the trophocytes become competent to synthesise Vg, has been described in the studies conducted with *Ae. aegypti*. During this phase (the 1st 3 days after hatching), enlargement and activation of the nucleoli, proliferation of the ribosomes and RER, development of Golgi complexes and extensive invaginations of the plasma membrane occur (Raikhel & Lea 1983). In *Cx. quinquefasciatus*, we did not observe this preparatory phase or activation of the biosynthetic machinery prior to a blood feeding. Moreover, we did not detect Vg synthesis or accumulation earlier than 12 h PBM. This physiological discrepancy, which is evidenced by morphological observations, may determine the chronological differences between the two mosquito species. Vg synthesis was observed by immunofluorescence at 3 h PBM in *Ae. aegypti* trophocytes (Raikhel & Lea 1983) whereas in *Cx. quinquefasciatus*, Vg synthesis begins at approximately 12 h PBM as evidenced by immunoblotting and the presence of only a few, small RER cisternae. In *Cx. quinquefasciatus*, the emergence of electron-dense vesicles and granules from the Golgi complex of the trophocytes was not observed earlier than 24 h PBM.

In *Ae. aegypti*, the trophocyte ultrastructure indicates a decline in Vg synthesis at 30-40 h PBM when the RER and Golgi complex are reduced and the secretion granules disappear (Behan & Hagedorn 1978, Raikhel & Lea 1983). In *Cx. quinquefasciatus*, the reduction of the biosynthetic organelles begins between 48-72 h PBM. Only at 84 h PBM do the trophocytes of *Cx. quinquefasciatus* return to the appearance that they had before the blood meal, 14 h later than the time described for *Ae. aegypti* (Behan & Hagedorn 1978).

The general structure of the *Cx. quinquefasciatus* ovaries is similar to that of other mosquitoes. Nonetheless, we observed morphological differences and some discrepancies in the temporal patterns of development between *Cx. quinquefasciatus* and *Ae. aegypti*. Several studies observed that while *Ae. aegypti* was still in its previtellogenic phase, oocyte differentiation was initiated and it became distinguishable from the other cells due to its smaller nucleus with a prominent nucleolus and the presence of lipid inclusions in its cytoplasm (Roth & Porter 1964, Raikhel & Lea 1983, Clements & Boocock 1984). This initial differentiation does not occur in pre-engorged *Cx. quinquefasciatus*, whose ovarian follicles consist of a set of eight undifferentiated cells before the first blood meal.

The uptake of Vg by oocytes is a receptor-mediated, clathrin-dependent endocytosis process (Roth & Porter 1964, Raikhel 1984, Raikhel & Dhadialla 1992). Accordingly, during the vitellogenic phase, coated vesicles are visible in the peripheral cytoplasm of *Cx. quinquefasciatus* oocytes.

Lipid and carbohydrates also accumulate in the developing oocytes. While lipid storage occurs with Vg uptake, carbohydrate accumulation is only evident at the

end of oogenesis. Briegel et al. (2003) described that in *Ae. aegypti* the deposition of glycogen is delayed when compared with vitellin and lipids. Van Handel (1992, 1993) also described a delayed accumulation of glycogen in *Cx. quinquefasciatus* and showed that glycogen is the only reserve that will be used by the mature oocytes to maintain their own integrity during the period between the chorionation and the oviposition.

Mosquito follicular cells are responsible for the synthesis and secretion of the eggshell components into the perioocytic space (Raikhel & Lea 1991, Lucantoni et al. 2006). The eggshell is an important structure that protects the oocyte and/or the developing embryo from a series of environmental hazards (Yao & Li 2003). Eggshell protein synthesis and secretion by the follicular cells is hormonally controlled by 20-hydroxyecdysone (Raikhel & Lea 1991, Edwards et al. 1998) and occurs in two phases, which results in two distinct layers called the endochorion and exochorion (Hinton 1968, Monnerat et al. 1999).

In *Cx. quinquefasciatus*, the follicular cells showed no morphological indications of synthetic activity before the engorgement and throughout the first 18 h PBM. Morphological changes begin to occur at approximately 24 h PBM with an increase in RER cisternae. At 48 h PBM, electron-dense plaques accumulate in the perioocytic space between the follicular cells and the oocyte and may represent the secretion of components of the endochorion layer. Later, at 60 h PBM, small electron-dense vesicles can be observed in the apical region of the follicular cells of *Cx. quinquefasciatus*. These vesicles seem to merge and form large dense granules, which are presumably precursors of the exochorion. Degeneration of the follicular cells occurs in a process that spans from 60 h PBM to approximately 84 h PBM, at which time they are completely degenerated. Follicular cell degeneration determines the end of the oogenesis process.

In conclusion, we have presented a morphological description of two organs involved in the process of egg formation in the mosquito *Cx. quinquefasciatus*, the fat body and the ovaries. We have also correlated the morphology with the protein profiles of the both organs. Comparisons between our observations and similar descriptions previously presented for the mosquito *Ae. aegypti* revealed a number of similarities and differences. With this study, we hope to contribute further knowledge of the physiology of this important, cosmopolitan and urban plague, the mosquito *Cx. quinquefasciatus*.

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