The ultrastructure of oogenesis in *Culex theileri*

S.R. Pollard, M.A. Motara and R.H.M. Cross

Department of Zoology and Entomology, Rhodes University, Grahamstown; Department of Zoology, University of Durban-Westville, Durban and Electron Microscopy Unit, Rhodes University, Grahamstown

The 12 – 36 h post blood meal (PBM) oocyte in *C. theileri* is characterized by a dramatic increase in the quantity of protein with concomitant changes in the adjacent follicular epithelium. The cytoplasm shows a preponderance of mitochondria and rough endoplasmic reticulum in proportion to free ribosomes. Numerous Golgi complexes, which bud off secretory granules, are also found scattered in the cytoplasm. These changes are concerned with the deposition of the vitelline membrane and by 36 h PBM, a continuous vitelline membrane is formed at the follicle cell – oocyte interface. Vitellogenesis appears to be completed by 48 h PBM and the final maturation stages are concerned solely with deposition of the chorionic membrane. In *C. theileri* the chorionic membrane seems to be synthesized in a similar manner to the vitelline membrane; namely from the aggregation of secretory droplets and only proceeds once the vitelline membrane formation is completed. The chorionic membrane is made up of an endochorion and an exochorion. Although the structural complexity of the follicular epithelium is maintained at 48 h PBM, its appearance at 60 h PBM is indicative of degenerative changes. By 60 h PBM synthesis of both the vitelline and chorionic membranes has been completed and vitellogenesis has ceased. This stage then represents the complete maturation of the ovum in *C. theileri*.
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Die oösiet van *C. theileri* is 12 tot 36 h na 'n bloedmaal (NBM) gekenmerk deur 'n dramatiese vermeerdering in die hoeveelheid proteïen met saamgaande veranderinge in die aangrensende follikulêre epiteel. Die sitoplasma bevat 'n oorwig van mitokondrië en growwe endoplasmiese retikulum in verhouding tot vrye ribosome. Veelvuldige Golgi-komplekse wat afskeidingskorrels afbot word verspreid in die sitoplasma gevind. Hierdie veranderinge is betrokke by die neerlegging van die dooiermembraan en teen 36 h NBM het 'n onafgebroke dooiermembraan by die skeidingsvlak tussen die follikulêre sel en die oösiet gevorm. Dooiervorming skyn 48 h NBM voltooid te wees en die finale ryp stadia is alleenlik betrek by die neerlegging van die korioniese membraan. Die korioniese membraan by *C. theileri* word vermoedelik op 'n soortgelyke manier as die dooiermembraan gevorm; d.w.s. deur die samevoeging van afskeidingsdruppels terwyl die neerlegging van die korioniese membraan alleenlik voortgaan wanneer dooiermembraanvorming voltooi is. Die korioniese membraan bestaan uit 'n endokorion en 'n eksokorion. Alhoewel die strukturele kompleksiteit van die follikulêre epiteel by 48 h NBM behou is, is die voorkoms van hierdie epiteel 60 h NBM aanduidend van degeneratiewe veranderinge. Teen 60 h NBM is beide die dooier- en korioniese membrane gevorm en dooiervorming alreeds voltooi. Hierdie ontwikkelingstadium verteenwoordig dan die ten volle ryp ovum by *C. theileri*.
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S.R. Pollard
Department of Zoology and Entomology, Rhodes University, Grahamstown, 6140 Republic of South Africa

M.A. Motara*
Department of Zoology, University of Durban-Westville, Private Bag X54001, Durban, 4000 Republic of South Africa

R.H.M. Cross
Electron Microscopy Unit, Rhodes University, Grahamstown, 6140 Republic of South Africa

*To whom correspondence should be addressed

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Oogenesis refers to a period of growth and formation of the oocyte and in mosquitoes is stimulated by the ingestion of a vertebrate blood meal (Chapman 1982). Vitellogenesis proceeds concurrently with oogenesis and represents the period of deposition of the yolk in the oocyte. In Diptera, vitellogenin, a unique group of proteins synthesized extra-ovarially, are only synthesized after the ingestion of a vertebrate blood meal (Hagedorn 1974). While oogenesis in mosquitoes has been studied (Anderson & Spielman 1971; Mathew & Rai 1975; Roth & Porter 1964; Tadkowski & Jones 1979), *Culex theileri* (Theobald), has not been investigated in this respect. Here we report the ultrastructural changes that occur in the oocyte and the surrounding follicular epithelium of *C. theileri* during oogenesis. Whether interactions within the follicular epithelium are essential for the progression of vitellogenesis was also addressed. Finally, since they appear to impart a specific protective function to the mature egg, the structure of the fully developed vitelline and chorionic membranes was also studied.

Materials and Methods

Virgin *C. theileri* females, obtained from Bloemfontein in the Orange Free State, South Africa were used in this study. They were maintained at a constant temperature of 27°C. The female mosquitoes were given an avian blood meal, and ovaries were dissected from a sample of the study population (n = 200) 12, 24, 36, 48, and 60 h post blood meal (PBM). The ovaries of unfed mosquitoes acted as controls.

The dissected ovaries were fixed in cold buffered glutaraldehyde (2,5% glutaraldehyde in 0,1 mol dm^{-3} phosphate buffer, pH 7,3 at 4°C) for 48 h. The tissue was rinsed twice in 0,1 mol dm^{-3} phosphate buffer before transferring it to 1% osmium tetroxide. It was allowed to fix in the latter for 2 h and again washed in phosphate buffer for 15 min.

After fixation, the tissue was dehydrated for 5 min in each of the following ascending concentrations of ethanol (30%, 50%, 70%, 80%, 90%, 100%, 100%). Thereafter, for 15 min each, the material was washed twice in propylene oxide.

For embedding in a mixture of TAAB-812/Araldite, the tissue was infiltrated with resin by being passed through a series of propylene oxide and resin mixtures (3:1, 1:1, 1:3). Owing to initial poor resin penetration, infiltration in each mixture was prolonged from 30 to 120 min. The material was transferred to pure resin for 14 h with the specimens finally being placed in resin on flat moulds and polymerized at 60°C for 36 h.

Sections were obtained using an LKB UM III ultramicrotome. For light microscopy thick sections of 3 µm were stained

with toluidine blue. For electron microscopy 60 nm sections (gold interference colour) were collected on 200 and 300 mesh grids, dried and stained with 5% aqueous uranyl acetate for 30 min and lead citrate for 4 min. A Jeol JEM 100CX II electron microscope was used for observation and photography.

Results

The ovaries of mosquitoes lie between the 4th and 6th abdominal segments and comprise 50–60 ovarioles of the meroistic polytrophic type. Each egg follicle consists of seven nurse cells and the oocyte surrounded by follicular epithelium. When fully ripe, the oocyte passes to the oviduct after the rupture of the follicular epithelium.

The gross morphological changes of the oocytes of *C. theileri* during oogenesis are shown in the composite light micrograph sections (Figure 1). A striking increase in oocyte size is seen between the pre-blood meal stage and 60 h PBM, from approximately 60 µm to 200 µm respectively, accompanied by an elongation of the oocyte. A characteristic of oocyte development is deposition of yolk within the oocyte which reaches a peak at 60 h PBM (Figure 1).

Control

In previtellogenic and vitellogenic ovarian follicles, the follicular epithelium consists of a uniform layer of cuboidal cells that surround the nurse cells and oocyte. During the pre-blood meal phase (Figure 2), the adjoining membranes which are closely applied at the apical membranes, dilate to form intercellular spaces of varying diameters at irregular basal locations. The cytoplasm contains many free ribosomes and mitochondria. Golgi complexes are sparse and no rough endoplasmic reticulum is discernable. Furthermore, the egg is completely filled with nurse cells interspersed with lipid vacuoles (Figure 2). The nurse cells display characteristics of metabolically active tissue by the presence of numerous quantities of glycogen, scattered mitochondria and Golgi complexes.

12 h PBM

Following the ingestion of blood by the mosquito, the appearance of the oocyte changes drastically. The follicle cells form a continuous layer surrounding the oocyte (Figure 3) and they remain in close apposition to the oocyte throughout vitellogenesis. Intercellular spaces filled with flocculent material

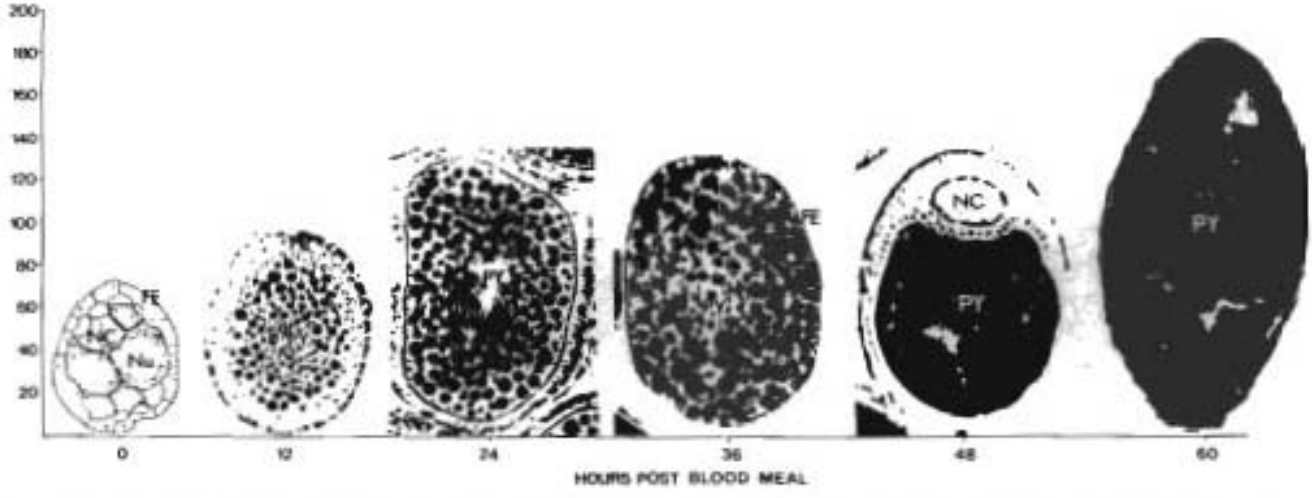

Figure 1 Light micrograph showing the gross progressive development of the *C. theileri* egg from the pre-blood meal to 60 h PBM stage. FE = follicular epithelium; NC = nurse cell; Nu = nucleus; PY = protein yolk.

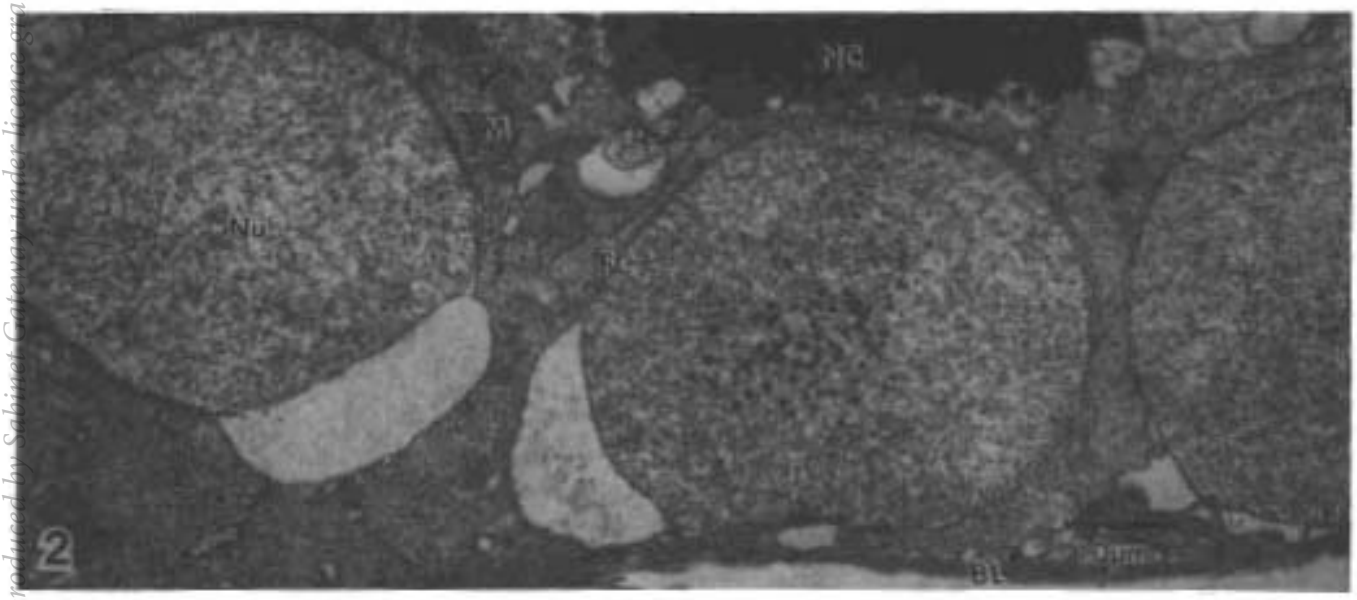

Figure 2 Micrograph of follicular epithelium, peripheral basement lamina and adjacent nurse cells of the pre-blood meal (control) egg.

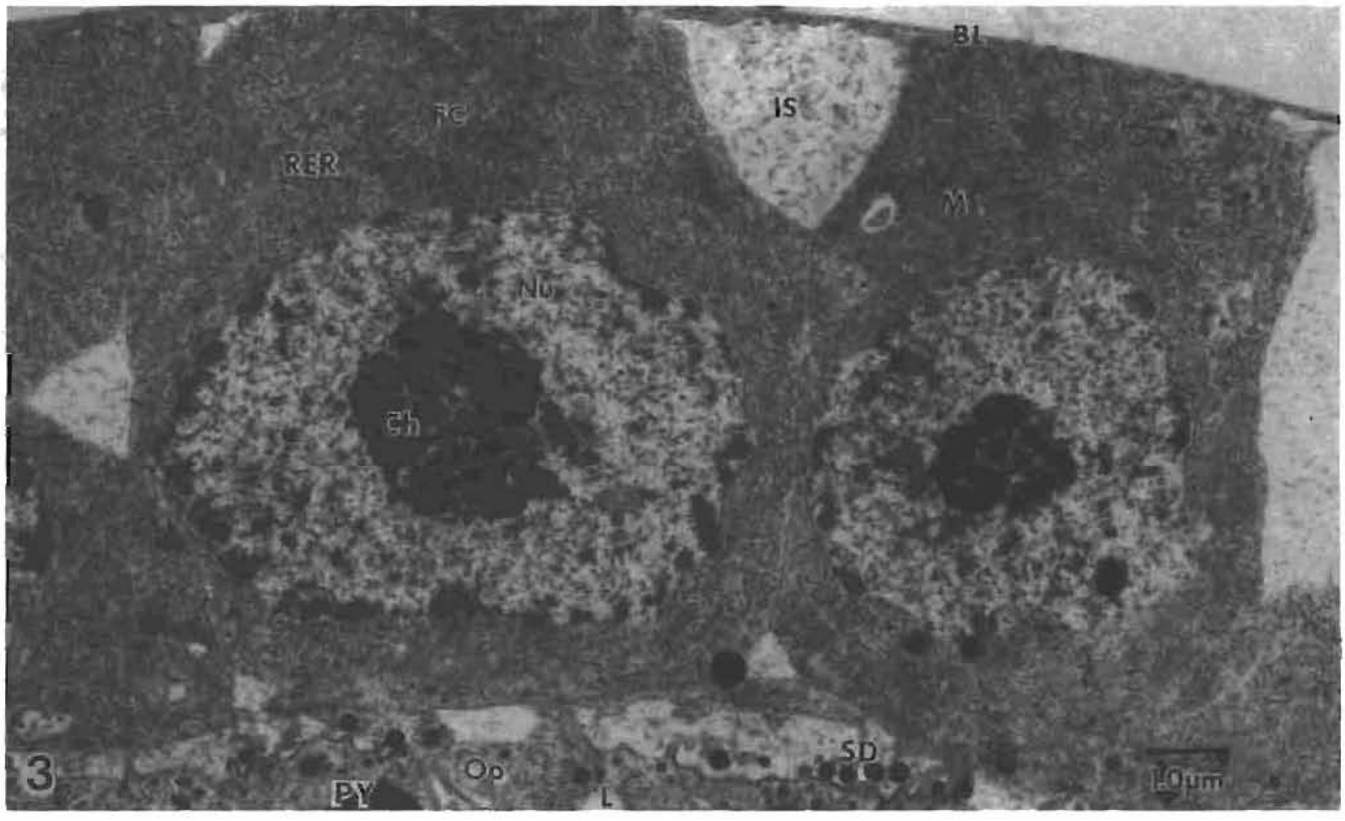

Figure 3 Micrograph of follicular epithelium and adjacent oocyte 12 h PBM.

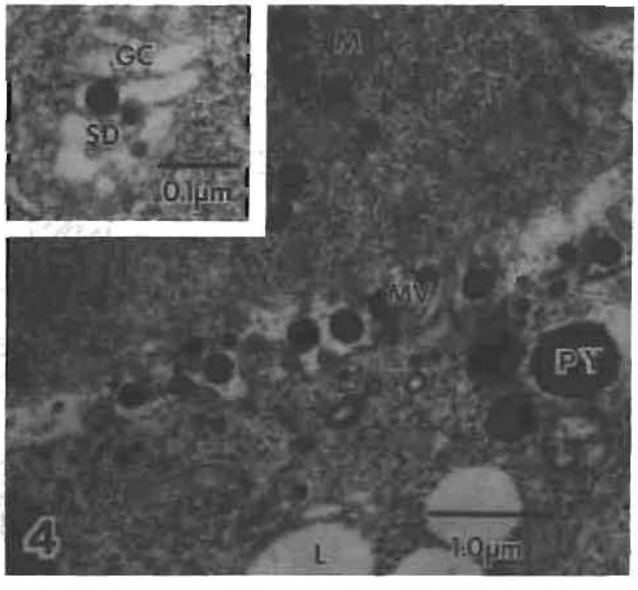

Figure 4 Early stages of vitelline membrane formation, 12 h PBM. Inset: Golgi complex and associated secretory droplets. BL = basement lamina; Ch = chromatin; FC = follicle cell; GC = Golgi complex; IS = intercellular space; L = lipid; M = mitochondria; MV = microvilli; NC = nurse cell; Nu = nucleus; Oo = oocyte; PY = protein yolk; RER = rough endoplasmic reticulum; SD = secretory droplets.

are prominent and a characteristic of this stage, and in some instances these spaces extend from the basement lamina to the oocyte surface. There is also an increase in the complexity of the fine structure of the cytoplasm with the appearance of rough endoplasmic reticulum and Golgi complexes and an increase in the number of mitochondria (Figures 3 & 4). Golgi complexes containing budding of electron dense secretory droplets can also be seen (Figure 4). They probably contain vitelline membrane precursors and aggregate between the microvilli of the oolemma at the follicle cell – oocyte interface. Microvilli proliferate from the surface of the oocyte and pinocytic vesicles are evident in the oolemma (Figure 4). Yolk deposition within the oocyte proceeds concurrently with vitelline membrane formation, and yolk droplets are initially detected in conjunction with small amounts of lipid droplets.

24 h PBM

Intercellular spaces are far less apparent at this stage but there are no substantial alterations in the composition of the cytoplasm of the follicle cells (Figure 5). Their apical cell membranes are occluded by electron-dense material which may represent extra-follicular precursor material. The secretory droplets have coalesced at the follicle cell – oocyte interface to form electron-dense plaques which compress the microvilli between them (Figure 6). There has been a progressive stocking of the oocyte with both protein yolk and lipid droplets.

36 h PBM

The most prominent development at this stage is the coalescing of the vitelline plaques to form a continuous homogenous layer, about 1,2 µm thick, which completely encompasses the oocyte (Figure 7). The follicle cells still contain abundant rough endoplasmic reticulum and mitochondria, but few Golgi complexes are seen. Small intercellular spaces dilate the basal cell membrane and it is tentatively suggested that desmosomes occlude the apical cell membrane (Figure 7).

48 h PBM

By 48 h PBM the follicular epithelial cells have changed in their overall shape from cuboidal to squamosal and the elongated nucleus occupies the major portion of the cell volume (Figure 8). Dense aggregations of rough endoplasmic

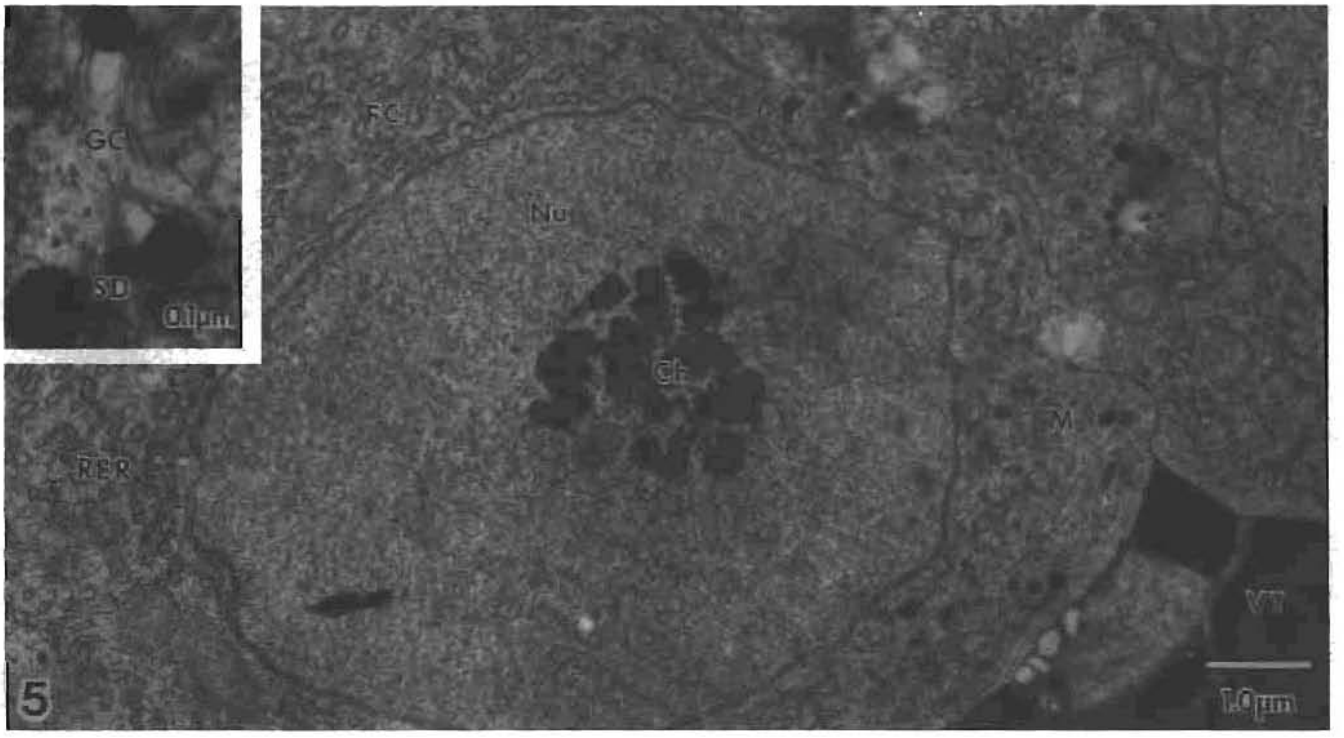

Figure 5 Micrograph of the follicular epithelium and adjacent vitelline membrane 24 h PBM. Inset: Golgi complex and associated secretory droplets.

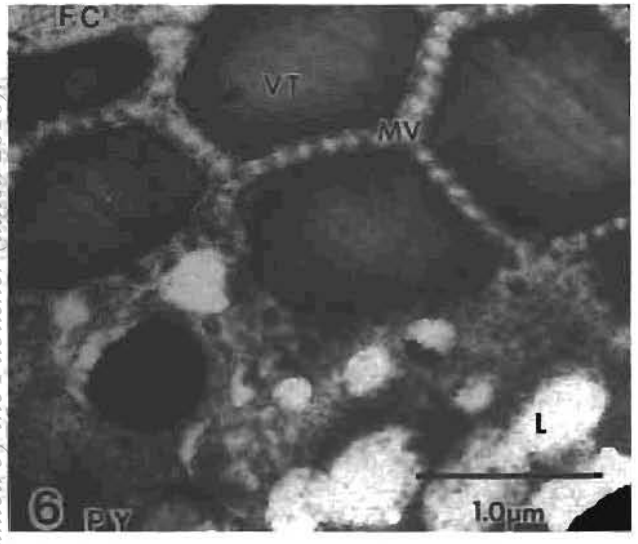

Figure 6 Vitelline membrane formation at the follicle cell – oocyte interface 24 h PBM.

reticulum and mitochondria are found in the cytoplasm and desmosomes are evident at the apical cell membrane. The chorionic membrane is visible for the first time (Figure 8). The initial stages of chorionic membrane formation proceed only once vitelline membrane formation is complete (36 h PBM). It appears from Figure 9 that the chorionic membrane is formed from secretory droplets in a fashion similar to the formation of the vitelline membrane and possibly from additional material deposited via the cell membrane. Although the detailed timing for chorionic membrane synthesis could not be established it is suggested that the lamellate membrane is the first to be synthesized (Figure 10), followed by deposition of the chorionic pillars and fibrous mesh. These structures then form the endochorion. The oocyte at this stage is filled with dense aggregations of yolk and lipid droplets (Figure 8).

60 h PBM

At this stage, the ultrastructural specializations preceding the secretion of the chorionic membrane are lost in the follicle cells. The decrease in size of the follicle cells and their poorly distinguished nuclei indicate a degenerative process in the follicular epithelium (Figure 11). The fine structure of the chorionic membrane is shown in Figure 11 and appears to be in the final stages of development since the exochorion has been laid down peripherally to the endochorion. A fibrous mesh is evident in association with the exochorion.

Owing to artifacts resulting from poor resin penetration, the vitelline and chorionic membranes appear to be separated from one another at both 48 and 60 h PBM (Figure 8). They are, however, found in close apposition.

Table 1 summarizes the changes that occur, both in the follicular epithelium and the oocyte, during oogenesis.

Discussion

General features

In the yellow fever mosquito *Aedes aegypti*, the oocytes of the newly emerged adult appear to possess no yolk (Tadkowski & Jones 1979). Both the light and electron micrographs confirm the lack of yolk in *C. theileri*, characterized by the presence of well defined nurse cells which fill the oocyte. Unlike *A. aegypti*, in which there is little change in the size of the oocyte up to 36 h PBM, the most dramatic increase in oocyte size in *C. theileri* occurs within 36 h PBM and is accompanied by a concomitant increase in the amount of protein yolk and lipid droplets. The developmental period from 36 to 60 h PBM is characterized by a progressive stocking of the ooplasm with protein and lipid, and the oocytes which were initially spherical start to narrow, assuming the shape of mature oocytes. Thus, central to the development of the oocyte, is the increase in protein yolk, formed by the conversion of vitellogenins by the developing oocyte. Vitellogenins are synthesized and released by the abdominal fat bodies which reach a peak between 28 and 36 h PBM (Hagedorn & Kunkel 1979).

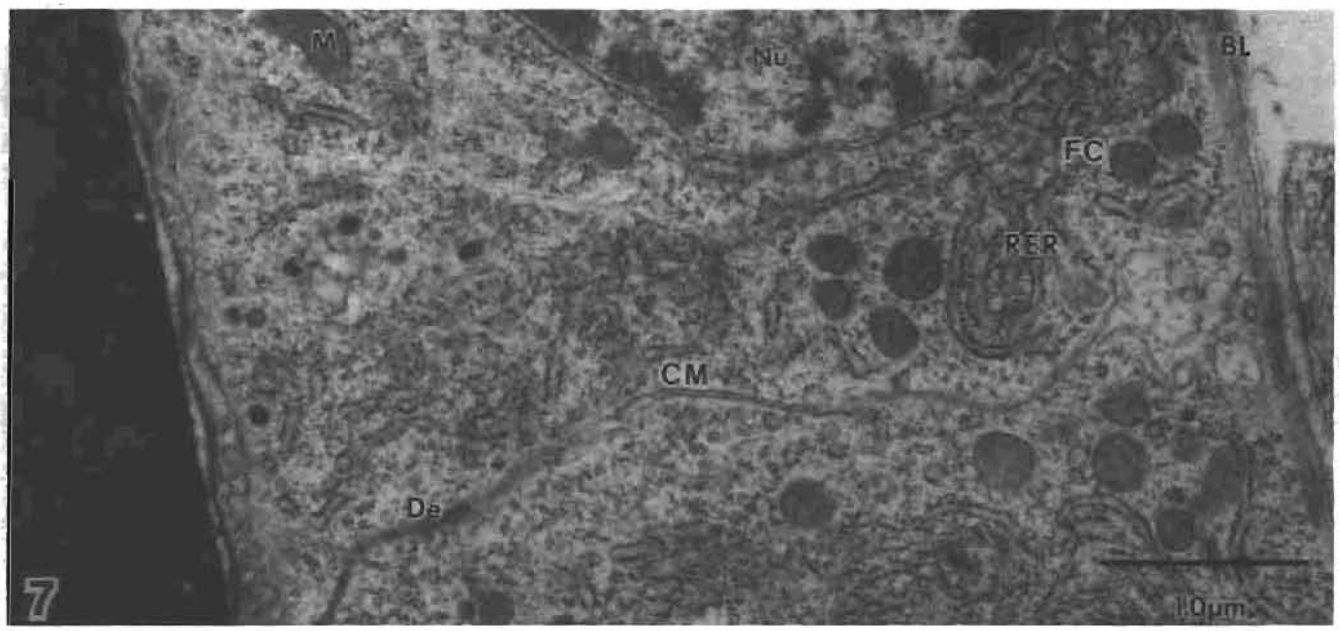

Figure 7 Micrograph of the follicular epithelium and adjacent vitelline membrane 36 h PBM. BL = basement lamina; Ch = chromatin; CM = cell membrane; De = desmosomes; FC = follicle cell; GC = Golgi complex; L = lipid; M = mitochondria; MV = microvilli; Nu = nucleus; PY = protein yolk; R = ribosomes; RER = rough endoplasmic reticulum; SD = secretory droplets; VT = vitelline membrane.

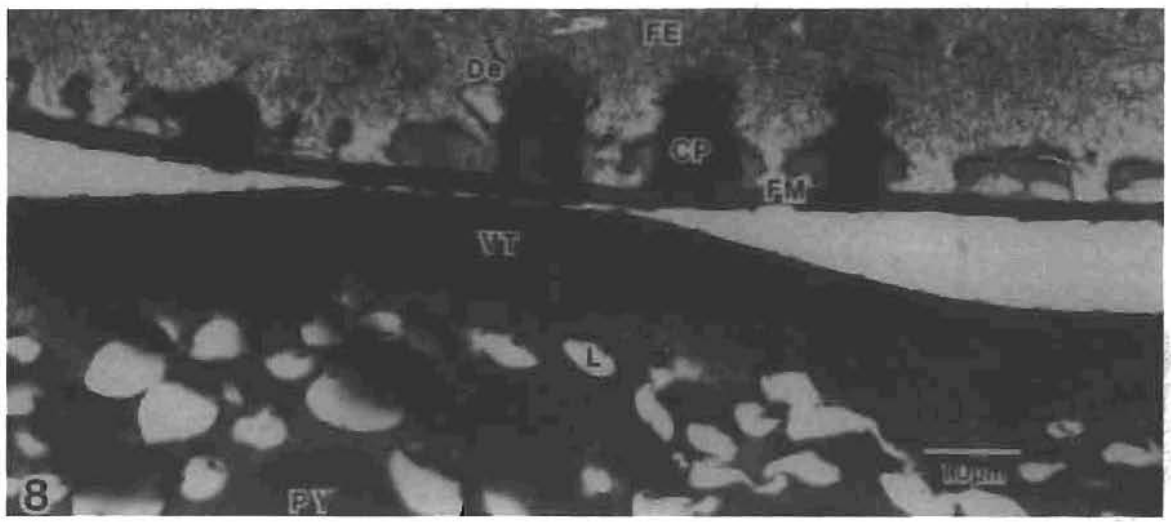

Figure 8 Micrograph of the oocyte and adjacent follicular epithelium 48 h PBM.

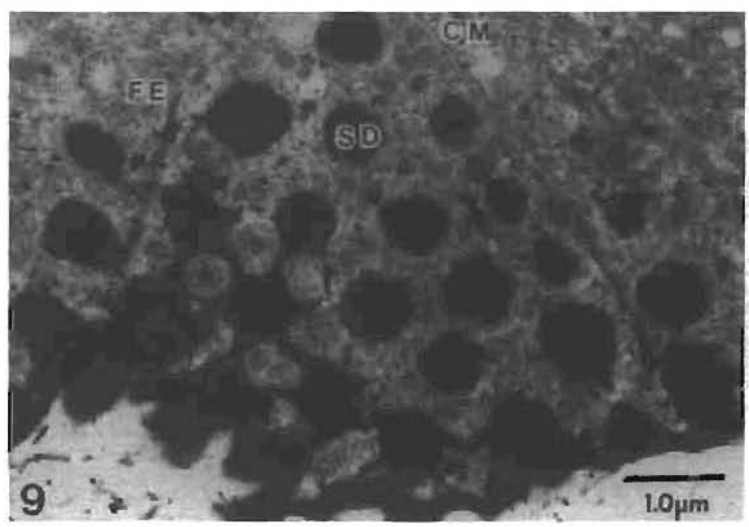

Figure 9 Micrograph showing the formation of chorionic pillars by coalescing of secretory droplets 48 h PBM.

Follicular epithelium and changes in its organization

The most important role of the follicular epithelium during insect oogenesis has classically been the deposition of the egg envelopes during choriogenesis (Junquera 1983). The follicle cells also contribute to the development of the oocytes (Anderson & Telfer 1969). However, controversy exists regarding their association in relation to the origin of the vitelline and chorionic precursors. Most studies tend to support their involvement in vitelline and chorionic membrane synthesis (King & Koch 1963; Anderson & Spielman 1971; Mathew & Rai 1975; Norton & Vinson 1982), but in at least one instance the oocyte itself is implicated in this process of vitelline membrane formation (Hopkins & King 1966).

Several morphological and cytological features indicate that the follicle cells are engaged in the deposition of the vitelline and chorionic membrane in the mosquito, *C. theileri*. In control animals, prior to the formation of the membranes the follicle cells are abundantly supplied with free ribosomes which are most common in undifferentiated cells and in cells synthesizing proteins from endogenous substances (Norton & Vinson 1982). At the onset of vitelline membrane formation, the follicle cells still possess many free ribosomes, but rough endoplasmic reticulum, which previously was sparse, increases significantly in quantity. Rough endoplasmic reticulum is characteristic of cells that secrete protein products (Carr & Toner 1982). Thus it appears that the follicle cells undergo

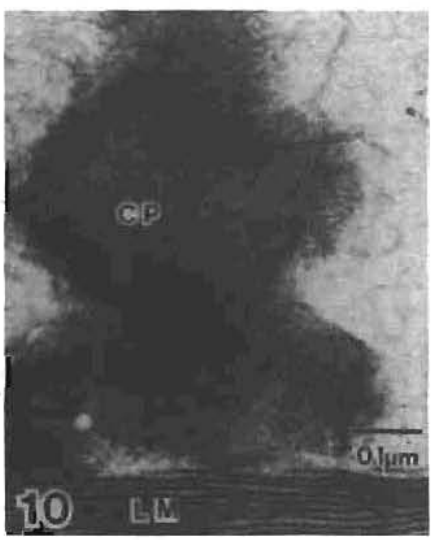

Figure 10 High power micrograph of the chorionic pillar and associated lamellate membrane 48 h PBM.

Table 1 Summary of transformations in the *C. theileri* follicle during oogenesis

	Hours post blood meal					
Structures	0	12	24	36	48	60
Follicular epithelium						
Intercellular spaces	++	++	+	+	−	−
Rough endoplasmic reticulum	−	+	++	++	++	−
Mitochondria	+	++	++	++	+	−
Golgi complex	−	++	++	+	−	−
Secretory droplets						
Vitelline	−	++	+	−	−	−
Chorionic	−	−	−	+	++	−
Vitelline membrane	−	−	+	++	++	++
Chorionic membrane						
Endochorion	−	−	−	−	++	++
Exochorion	−	−	−	−	+	++
Oocyte						
Nurse cells	++	+	+	+	+	−
Lipids	+	+	++	++	++	++
Protein yolk	−	+	++	++	++	++
Microvilli	−	++	+	−	−	−
Pinocytotic vesicles	−	++	+	−	−	−

− = Absent + = Present ++ = Abundant

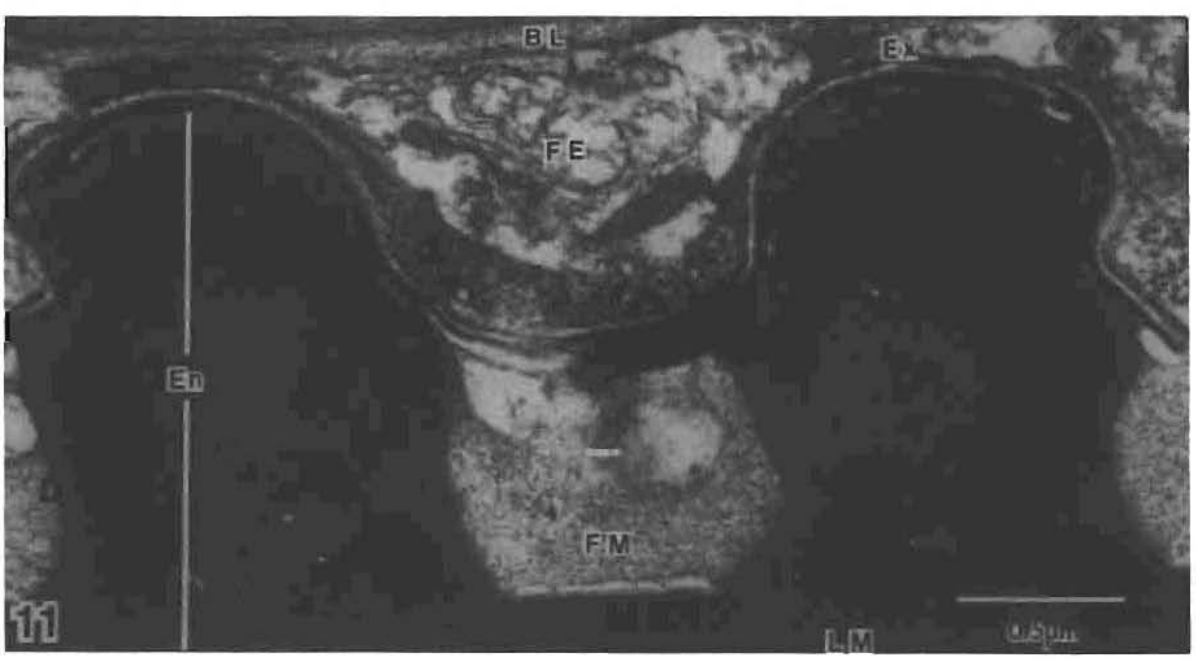

Figure 11 Micrograph of the chorionic membrane and the degenerating follicular epithelium 60 h PBM. CM = cell membrane; CP = chorionic pillar; De = desmosomes; FE = follicular epithelium; FM = fibrous membrane; L = lipid droplet; LM = lamellate membrane; PY = protein yolk; SD = secretory droplets; VT = vitelline membrane; BL = basement lamina; En = endochorion; Ex = exochorion; FE = follicular epithelium; FM = fibrous membrane.

a structural change which results in the initiation of synthesis and secretion of the membranes. It is not possible, however, to completely disregard the possible involvement of the oocyte in membrane synthesis.

It is generally accepted that the Golgi complexes are involved in the formation of the secretory granules, which bud off from the Golgi cisternae. Mathew & Rai (1975) suggest that the endoplasmic reticulum is involved with the synthesis of vitelline precursors, from where they are transferred to the Golgi complex prior to the formation of the secretory granules. In view of the previous discussion regarding the increase in the quantity of rough endoplasmic reticulum, it is plausible that this is indeed the case in *C. theileri*.

At 12 h PBM the vitelline precursors are present in the follicular cells and at the epithelium – oocyte interface where they later coalesce to form plaques which subsequently compress the microvilli. The vitelline membrane appears as a continuous layer by 36 h PBM. Chorionic deposition has not been initiated at this stage but the chorionic membrane is clearly distinguished by 48 h PBM.

Classically it is accepted that the chorion is comprised of two layers, an outer exochorion and the inner endochorion (Hinton 1968). The literature regarding the identification of the endochorionic layers is contradictory. Mathew & Rai (1975) propose that the endochorion is a two-layered structure interconnected by vertical struts, the inner layer having the chorionic pillars and fibrous mesh-like areas and the outer having a fibrous layer only. Separating the endochorion from the vitelline membrane is a lamellate membrane. Tadkowski & Jones (1979) identify the 'fibrous mesh' as the exochorion in *A. aegypti*, implying that the endochorion is the outer layer of the chorionic membrane, whilst Mathew & Rai (1975) fail to identify the exochorion. We suggest that in *C. theileri* the endochorion is composed of the lamellate membrane and chorionic pillars which are separated by a fibrous mesh. The vertical struts referred to by Mathew & Rai (1975) were not observed in this study. Finally, at 60 h PBM, the chorionic pillars are covered in a thin electron-dense layer which we suggest represents the endochorion.

Controversy also prevails with regard to the manner in which the chorionic membrane is laid down. Mathew & Rai (1975) state that the plasma membrane in *A. aegypti* is thrown into folds resulting in channels through which the secretory droplets reach the follicle cell interface. No such channels were observed in this study, and from Figure 11 it seems far more plausible to suggest that chorionic pillars are laid down in a similar manner to the vitelline membrane: namely by coalescence of the secretory droplets. Furthermore, Mathew & Rai (1975) postulate that the fibrous membrane is formed by fibrous material synthesized by rough endoplasmic reticulum. No fibrous bodies were identified in this study and additional research on the composition of the fibrous mesh is necessary before any organelle(s) can be implicated in its formation.

The oocyte

A question that has frequently been addressed is which organelles are responsible for the formation of yolk proteins. Roth & Porter (1964) identified certain cells of the midgut of *A. aegypti* as having the structural equipment necessary for yolk synthesis. Tadkowski & Jones (1979) implicate the abdominal fat bodies as the major source of protein yolk, although conceding that the midgut may provide an additional source. Furthermore, it seems likely that a considerable amount of protein yolk is synthesized endogenously after deposition of the endochorionic plaques (Anderson & Spielman 1971). This is supported in *C. theileri* by observations of pinocytosis by the oolemma being prominent solely in the early stages of vitellogenesis, suggesting that the site of yolk synthesis may shift from extra to intra-oocytic sites during vitellogenesis. Furthermore, in the later stages of development, the vitelline membrane presents a potential barrier to the inflow of exogenous material and the cell membranes of the follicle cells become occluded by desmosomes. In *A. aegypti* the basement lamina acts as a coarse mechanical filter allowing deposition of the yolk from exogenous material via intercellular channels. Subsequently, however, the intercellular channels are occluded by desmosomes and the follicular epithelium acts as an effective seal (Anderson & Spielman 1971).

Acknowledgements

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