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Egg Development in the Arabian Goat and Sheep Tick, *Hyalomma arabica* Pegram, Hoostraal and Wassef 1982 (Acari, Ixodidae)

Mohammed S. Al-Khalifa

Deprtment of Zoology, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia

ABSTRACT. The development of ovarian oocytes of the sheep and goat tick *Hyalomma arabica* Pegram, Hoostraal and Wassef 1982, has been investigated by the light and electron microscope. Such studies revealed that the egg development could arbitrarily be divided into previtellogenic and vitellogenic stages distinguishable under the light microscope. The ultrastructural changes during egg development are described. Profiles of endoplasmic reticulum and Golgi complex are active in yolk synthesis. As vitellogenesis proceeds, yolk precursors are incorporated into the egg by micropinocytosis at the egg surface. Thus, in *H. arabica,* yolk materials appear to be derived from both intra- and extra-oocytic sources. The mitochondria and lipids are abundant. Other cytoplasmic components are illustrated.

Among the arthropods, oogenesis is well documented in insects (Bonhag 1958; Telfer 1965; Norrevang 1968 and Anderson 1974), but apart from a new studies (Aeschlimann and Hecker 1967, 1969; Brinton and Oliver 1971; Diehl 1970; Khalil 1969, 1970 and Raikhel 1978), the process is little investigated in ticks. The Arabian goat and sheep tick, *Hyalomma arabica* Pegram, Hoogstraal and Wassef 1982, has been described recently from the Al-Sarawat Mountains of Yemen and Saudi Arabia (Pegram *et al.* 1982). Since then a few studies have been carried out on this tick mainly concerning its distribution, seasonal abundance and host range in Saudi Arabia (Al-Khalifa *et al.* 1986, 1987 and Diab *et al.* 1985).

In the present study oogenesis in the tick has been investigated using both light and electron microscopy.

Material and Methods

Ticks originally collected from goats and sheep at Al-Sarawat Mountains of

Makkah Province by Al-Khalifa *et al.* (1986) and Diab *et al.* (1985) were used to establish laboratory colonies at the Department of Zoology, King Saud University in Riyadh. These colonies are maintained on rabbits and *H. arabica* females were obtained for histological investigation. These were killed by ether, dissected in saline and their ovaries were fixed in either Carnoy's 6:3:1 or Bouin's fixative and processed for histological and histochemical studies. Thin (7 μm thick) paraffin sections were prepared and stained with Harris haematoxylin and eosin according to the method of Mallory (1944) (see Humason 1979). The pyronin-methyl green technique according to the method of Kurnick (1955) (see Pearse 1960) was used as general test for nucleic acids. For selective extraction, the sections were treated according to the method of Deane (1946) (See Casselman 1962).

For electron microscopy, the females were dissected in ice-cold 3% glutaraldehyde (manufactured by SERVA) fixative and their ovaries were immediately removed to fresh ice-cold 3% glutaraldehyde in 0.1M sodium cacodylate buffer and 0.17M sucrose at PH 7.4 where each was teased apart. They were left in glutaraldehyde fixative for periods of 90 min, after which they were washed (4-5 changes) in cacodylate buffer with 0.34M sucrose at 0-4°C overnight.

The ovaries were post-fixed in 1% osmium tetroxide in 0.1M sodium cacodylate and 0.17M sucrose at 4°C for 90 min. The tissues were embedded flat in pure Epon 812. Sections (75 nm thick) were cut on a Reichert ultramicrotome, stained with 30% uranyl acetate in methanol for 30 min and lead citrate for 7 min (Reynolds 1963), then examined under a Zeiss EM 9 S 2, operating at 60 kV.

Results

The *Hyalomma arabica* female has a single, horseshoe-shaped, tubular ovary that opens distally into the oviducts (Fig. 1). During oocyte development several processes were indicated and it was convenient to divide these processes into arbitrary phases. The first phase is previtellogenic where eggs with no evidence of yolk material were observed. These oocytes measure 28 × 42 μm. and are surrounded by a thin oolemma. At this stage the cell membrane is relatively smooth and without microvilli. The oocyte nuclei are large relative to the cell size and contain electron-dense evenly dispersed, flocculent material, presumably chromatin (Figs. 2 and 3). The oocytes gradually increase in size during the later stages of this phase. The ooplasm of these oocytes contains many free ribosomes and few mitochondria.

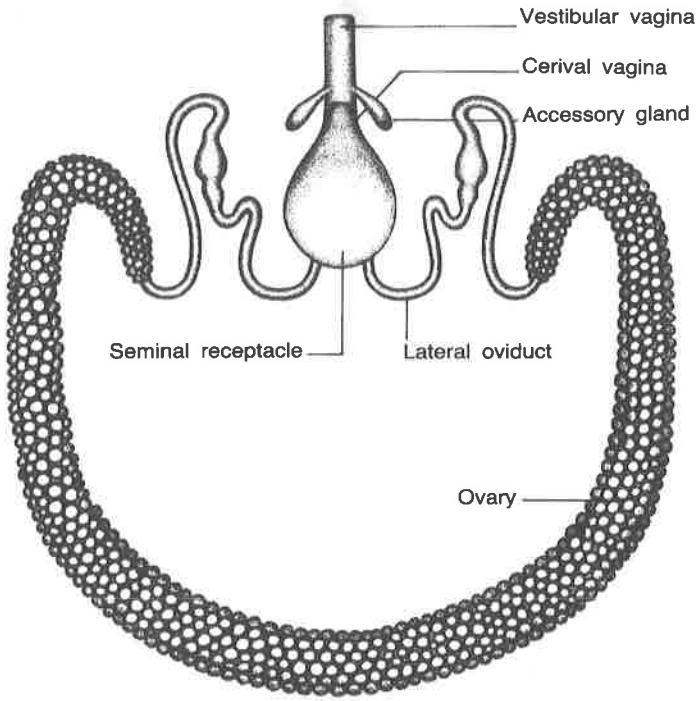

Fig. 1. Diagramatic representation of reproductive system of *Hyalomma arabica* from the dorsal aspects.

Coinciding with the onset of vitellogensis, the first changes are seen in the nuclei of the eggs, when large, fairly compact electron-dense inclusions appear. These are RNA-positive and similar inclusions were also observed near the pores of the nuclear envelope (Fig. 4). This could be nucleolar material being passed to the ooplasm and may account for the increase in number of ribosomes observed at this time. At the beginning of this phase, small fragments of endoplasmic reticulum appear. The Golgi complexes are apparently active and have numerous vesicles associated with them (Figs. 5 and 6). Few pinocytotic pits and vesicles were observed at the oocyte periphery during the early stages of this phase of vitellogensis (Fig. 8). During the later stages of this phase and at the appearance of vitelline membrane, more pinocytotic pits were observed at the oocyte periphery together with numerous vesicles (0.07-0.11 μm. in diameter), some of which are pinched off into the ooplasm (Figs. 9 and 11). These vesicles are partly or completely filled with electron-dense material. Their limiting membrane is bounded on its ooplasmic surface by an array of radiating rod-like structures. These vesicles lose their coats and fuse to form larger ones that contain fine, granular material (Figs. 9 and 11) and, later, large yolk precursor granules, which

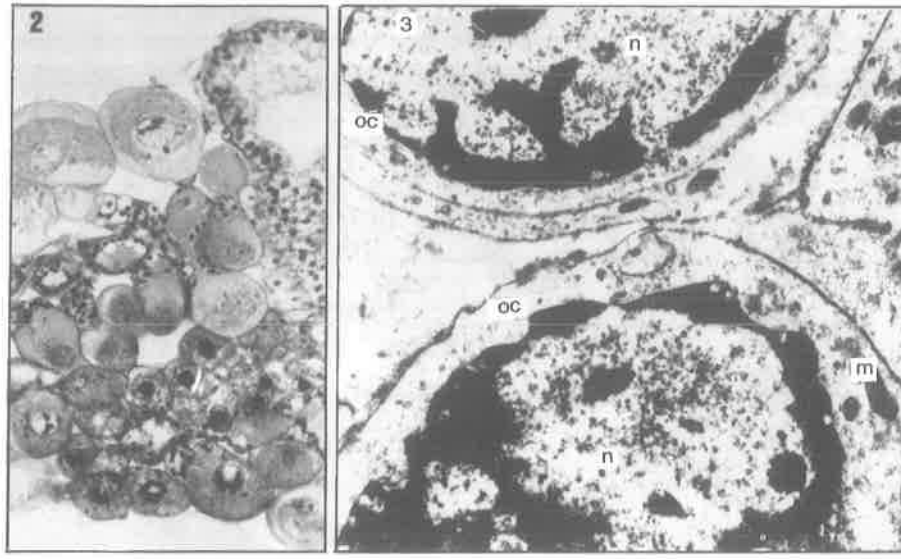

Fig. 2. A photomicrograph of the ovary in cross section showing several oocyte at previtellogenesis stages (arrow heads) and early vitellogenesis stages (arrows). 130 X.

Fig. 3. Electron micrograph of previtellogenic oocytes (OC) showing relatively large nuclei(n) with electron-dense inclusions. Mitochondrion(m). 11700 X.

probably fuse with autosynthetic granular precursors to produce mature yolk granules (Fig. 10). In the early vitellogenic phase the mitochondria are more numerous and are evenly distributed throughout the cytoplasm (Figs. 4 and 7). The lipid yolk droplets are distributed throughout the cytoplasm and aggregate occasionally to form groups (Fig. 6). These aggregations disappear with the increase of yolk formation, and the lipid droplets as well as the protein spheres become distributed throughout the ooplasm to occupy a considerable part of it (Fig. 10). However, it was not possible to trace the origin of lipid droplets.

During the late vitellogenic stage a vitelline envelope appears in between the microvilli borders of oocyte surface where micropinocytosis was observed to be very active. Towards the end of vitellogensis, the microvilli are located in narrow channels crossing the vitelline envelope that is produced by coalesced plaques (Fig. 11).

Discussion

As with other tick species (Khalil 1969, 1970; Roshdy 1969; Brinton and Oliver 1971 and Balashov 1983), the *H. arabica* female was observed in the

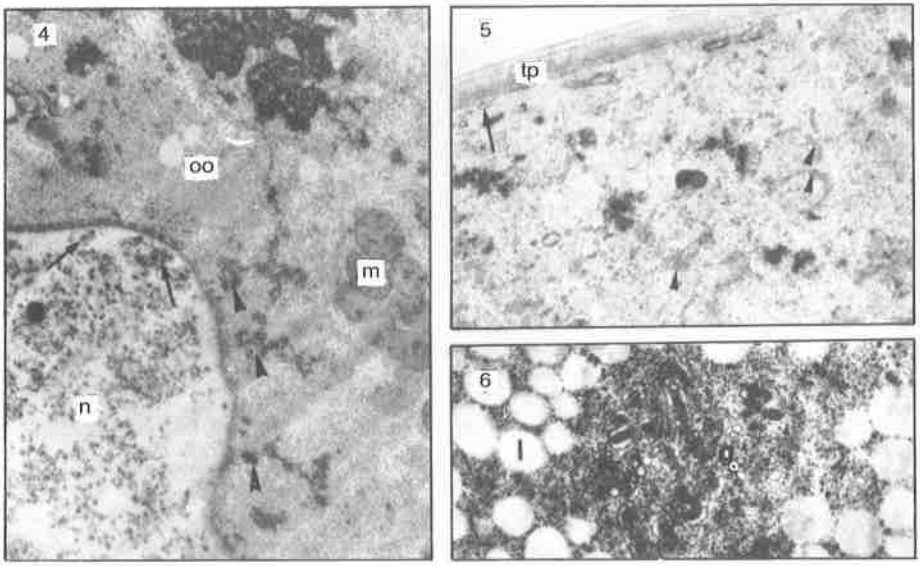

Fig. 4. Electron micrograph of early vitellogenic oocyte showing ooplasm with densly packed ribosomes(OO), nucleus(n) with electron-dense inclusion (arrows) and nuclar emission (arrow-heads). Mitochondrion(m). 22000 X.

Fig. 5. Electron micrograph of early vitellogenic oocyte showing ooplasm with several fragments of endoplasmic reticulum (arrow-heads). Note the early formation of microvilli at the surface of the oocyte cytoplasm (arrow). Tunica propria(tp). 10500 X.

Fig. 6. Electron micrograph of early vitellogenic oocyte showing large active Golgi complex (g) and lipid droplets(1). 18000 X.

present study to have a single horsehoe-shaped ovary.

The origins of the protein yolk in the oocyte of arthropods differ from one group to another. In some arthropods part of it is produced outside the ovary, carried via the blood to the developing follicles, then into the ooplasm by specialized pinocytosis, where it is deposited as yolk droplets: a process called heterosynthesis (Telfer 1965; Norrevang 1968 and Anderson 1974). In other groups there is evidence that the oocyte organelles are involved in yolk formation, a process designated as autosynthesis (Jarvis and King 1972, 1975). While in others both intra- as well as extra-oocyte processes have been observed in yolk formation (Aeschlimann and Hecker 1969, Brinton and Oliver 1971 and Raikhel 1978), which seems to be the method involved in *H. arabica.*

As far as the intra-oocyte (autosynthesis) formation of yolk in *H. arabica* is concerned, the present study suggests that the endoplasmic reticulum and Golgi

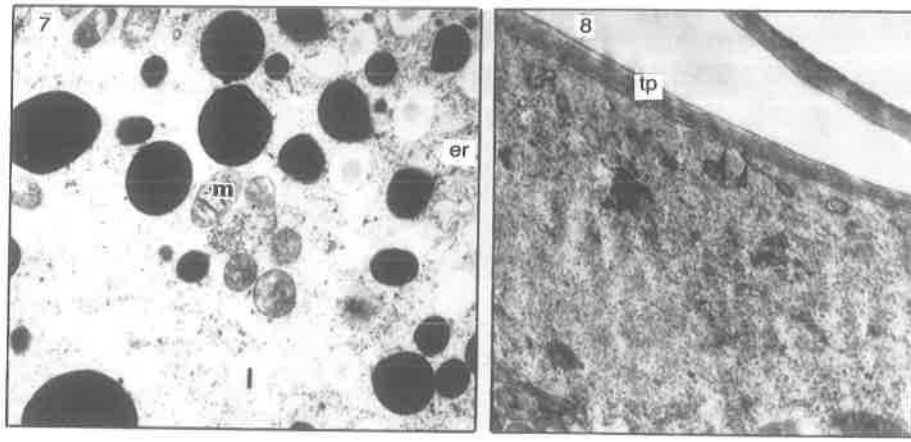

Fig. 7. Electron micrograph of vitellogenic oocyte showing expanded cisternae of endoplasmic reticulum(er) and yolk inclusion(y). Lipoid droplet(1) and mitochondrion(m). 22000 X.

Fig. 8. Electron micrograph of oocyte periphery showing early formation of microvilli (arrow-heads). Tunica propria(tp). 11700 X.

complex which were observed appear to be involved in these processes. The smooth membrane-bound vesicles of the Golgi complexes observed in the present study (Fig. 7) might become associated with the endoplasmic reticulum, and ultimately fuse with them forming a complex branching system of tubular and vesicular elements. Some of the small aggregations of medium electron-dense flocculent material observed may represent the initial stage of accumulating autosynthetic precursors for protein yolk. However, the endoplasmic reticulum was not as highly oriented in its spatial arrangement as that observed in the crayfish by Beams and Kessel (1963). Moreover, the involvement of the Golgi complex could hardly be determined with certainty from the present ultrastructural observations. However, such complexes were observed to be definitely involved in the vitellogensis of a Pycogonid (Jarvis and King 1972, 1975), although, micropinocytosis was more clearly discernible in extra-oocyte vitellogensis in *H. arabica* than in the Pycnogonid studied by Jarvis and King (1972, 1975). The channels observed in the late stage of vitellogensis in the present study might permit the passage and uptake of yolk protein precursors along with the deposition of the envelope. The exact origin of the materials incorporated into the yolk droplets of *H. arabica* oocyte was not traced in the present investigation.

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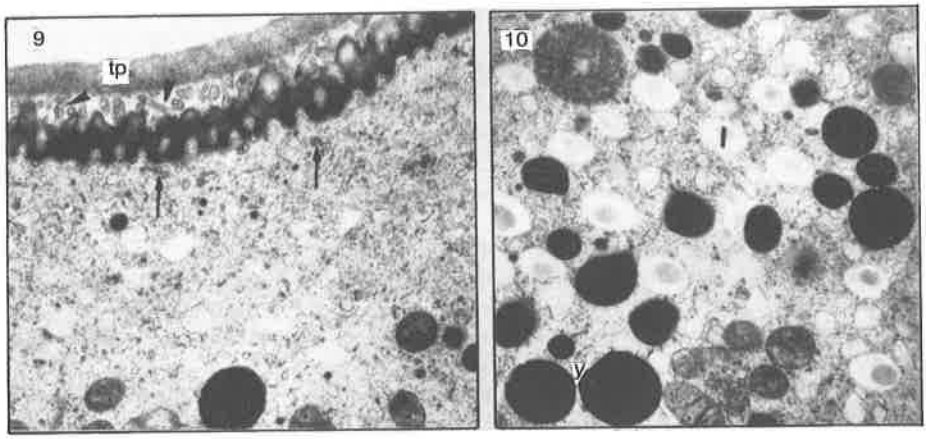

Fig. 9. Electron micrograph of oocyte showing well developed microvilli (arrow-heads) at the oocyte periphery. Note the activity of pinocytosis and the fusion of small pinocytotic vesicles (arrow-heads). Tunica propria(tp) 22000 X.

Fig. 10. Electron micrograph showing portion of oocyte cytoplasm during active vitellogenic phase. Note the enlarged yolk droplets(y) and lipoid droplet(l). Endoplasmic reticulum(er) and mitochondrion(m). 22000 X.

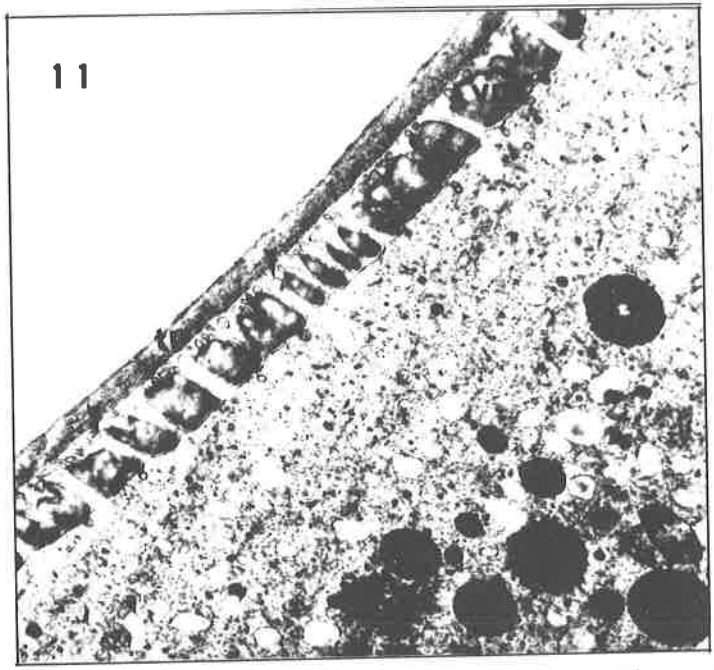

Fig. 11. Electron micrograph of oocyte during active vitellogenesis and vitelline envelope formation. Vitelline membrane(vm), tunica propria(tp); microvilli (arrow-heads) and yolk droplet(y). 22000 X.

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دراسة نمو البيض في مبيض قراد الماعز والضأن العربي

محمد صالح الخليفة

قسم علم الحيوان ـ كلية العلوم ـ جامعة الملك سعود ـ ص. ب ٢٤٥٥ الرياض ١١٤٥١
المملكة العربية السعودية

تم جمع القراد من الماعز والضأن من جبال السروات في منطقة مكة المكرمة والتي إستعملت لإستحداث مستعمرة حيوانية بقسم علم الحيوان ـ كلية العلوم ـ جامعة الملك سعود بالرياض.

تجمع إناث القراد للدراسة النسيجية والتركيب الدقيق ثم تقتل بالأيثر ومن ثم تشرح في محلول ملحي، حيث تنقل بعد ذلك المبايض إلى المثبت حسب نوع الدراسة فلقد إستخدم مثبت بوان للدراسات الهستولوجية ومثبت الجلوتر الدهيد ورابع أكسيد الأزميوم لدراسة التركيب الدقيق.

لقد درس البيض النامي في إناث قراد الماعز والضأن العربي بعد التغذية بأربعة أو ثمانية أيام باستعمال الطرق النسيجية والمجهر الألكتروني.

هذه الدراسة نستدل منها على أنه يمكن تقسيم نمو البيض إلى عدة أطوار من السهل تمييزها تحت المجهر الضوئي. ولقد دعمت دراسة التركيب الدقيق للأطوار المختلفة من نمو البيض صحة مثل هذا التقسيم ولقد لوحظ أن مجموعات من الشبكة الأندوبلازمية ومعقدات جولجي نشطة في تكوين المح عند تقدم عملية نمو البيض فإن هناك إضافة لمكونات المح تتكون نتيجة لحدوث عملية التشرب الخلوي الدقيق على طول سطح البيض وهذه المواد والتي تصل إلى البيض ربما ترد إلى المبيض من الهيموليف ولهذا يبدو أن مواد المح في قراد الماعز والضأن العربي تتكون من مصدر داخلي وآخر خارجي.

درست العضيات السيتوبلازمية الأخرى من أجسام سبحية وحبيبات الدهن وغيرها في البيض النامي.