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Structure of the ovariuterus of the scorpion *Euscorpius carpathicus* (L.) (Euscorpiidae) before fertilization

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Abstract
The ultrastructure of the ovariuterus of *Euscorpius carpathicus* (Scorpiones, Euscorpiidae) before fertilization was studied. Its wall presents a smooth muscular tunica and a pseudostratified columnar epithelium. At the level of the oocytes, the muscular wall is interrupted. The structure of the cells of the oocyte peduncle, issued from the epithelium, undergoes change. The vitellogenesis is divided into four steps, which are characterized by the size of the oocyte and by differences in the number and the structure of the cell organelles.

Key words: *Euscorpius carpathicus*, genital apparatus, ovariuterus, ultrastructure, vitellogenesis

INTRODUCTION

The different embryonic patterns of development allow the grouping of families in Scorpions. Three main patterns of development have been reported: within the tubules of ovariuterus (apoïkogenic), within specialized diverticula (katoikogenic) (Laurie 1890; Pawlowsky 1924, 1926) or in an intermediate and complex form of apoïkogenic ovariuterus (Farley 1996, 1998, 1999). *Euscorpius carpathicus* presents an apoïkogenic development. It is characterized by the oocytes, which are filled up with yolk, and by the development of embryos within the ovariuterine tubules. In this work, we study the ovariuterus ultrastructure of *Euscorpius carpathicus* females before fertilization.

MATERIAL AND METHODS

Adult female scorpions were caught in southern France. They were kept singly in bottles containing moistened leaf-mould at 26°C, and were fed young crickets.

Adult females were anaesthetized with chloroform. The genital tract was dissected and fixed with 2.5% glutaraldehyde in 0.2M cacodylate buffer (pH 7.3), containing 2.45% saccharose. Samples of the genital tract were rinsed in this buffer, then post-fixed with 1% osmium tetroxide and dehydrated. They were then embedded in epoxy resin. Semi-thin sections were stained with toluidine blue. Thin sections were stained with uranyl acetate and lead citrate and observed with a Philips electron microscope.

RESULTS

Anatomical study

The female reproductive system of *Euscorpius carpathicus* is composed of a tubular ovariuterus organized like rungs of a ladder, with three longitudinal tubules interconnecting with four pairs of transverse tubules (Pawlowsky 1926). It is prolonged at each side by a short oviduct and a seminal receptacle. The genital aperture is situated on the second opisthosomal segment.

The earliest immature germ cells are located

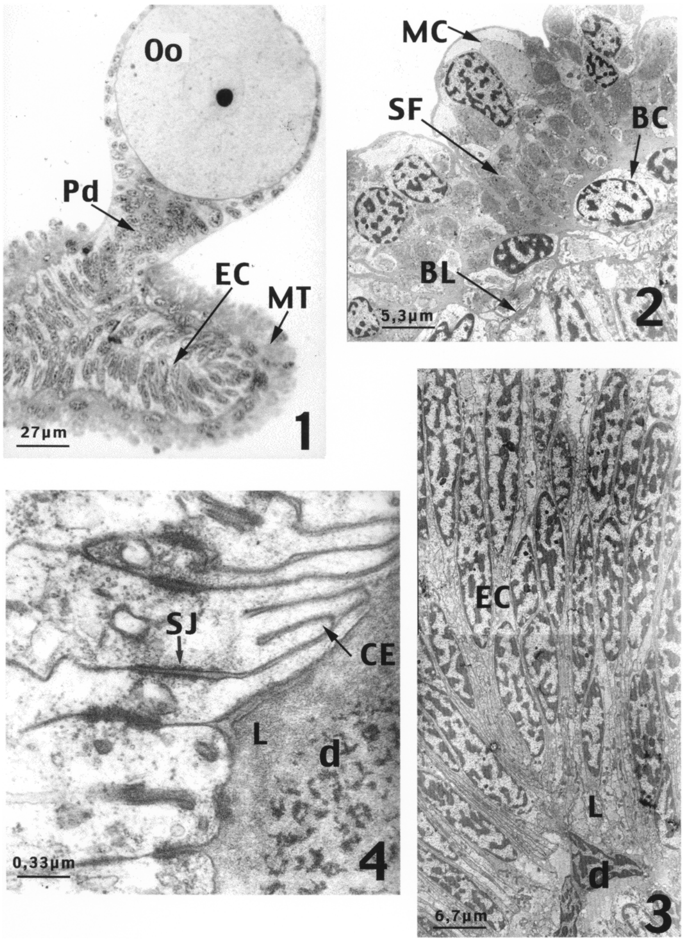


Fig. 1. Semi-thin section of the ovariuterus. EC: Epithelial Cells; MT: Muscular Tunica; Pd: Peduncle; Oo: Oocyte.
Fig. 2. Smooth muscular tunica. BC: Basal Cell; BL: Basal Lamina; MC: Muscular Cell; SF: Smooth fibers.
Fig. 3. Pseudostratified epithelium. d: degenerating epithelial nucleus; EC: Epithelial Cells; L: Lumen.
Fig. 4. Apical side of epithelial cells. d: degenerating epithelial nucleus; CE: Cytoplasmic Expansions; L: Lumen; SJ: Septate Junctions.

inside the wall of the ovariuterus (see histological study). Afterwards, they pass on the periphery of the ovariuterus and start to grow. During this period they are held by a peduncle (Pd, Fig. 1).

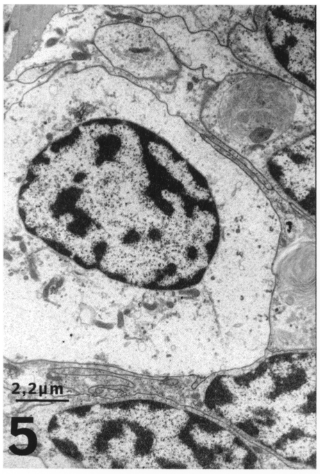
After fertilization, the embryos pass into the tubules and complete their development there in the normal manner for apoïkogenic scorpions.

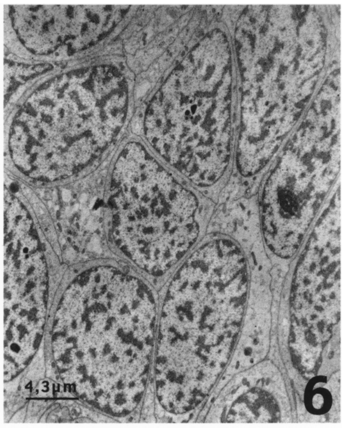
Ultrastructural study
The ovariuterus
The ovariuterus is circular in transverse section. Its wall is composed of a muscular tunica (MT) organized into several layers of smooth muscular cells (MC) (Figs. 1 and 2) and a pseudostratified epithelium (EC), lying on a basal lamina (BL). Connective tissue is well developed between muscular cells. The epithelium consists of very high columnar cells (EC), whose nuclei are elongated with a concentration of chromatin (Fig. 3).

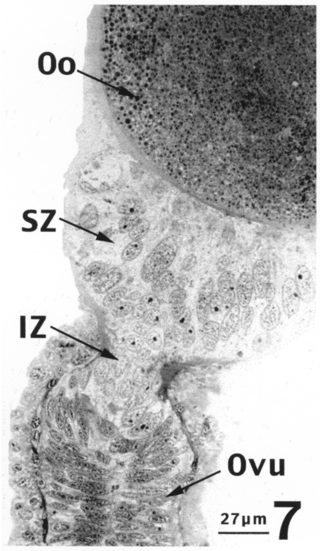
At the apical end of these cells are cytoplasmic expansions (CE) and septate junctions (SJ) (Fig. 4). The lumen (L) of the ovariuterus is narrow and often occupied by a flocculent material and degenerating epithelial nuclei (d).

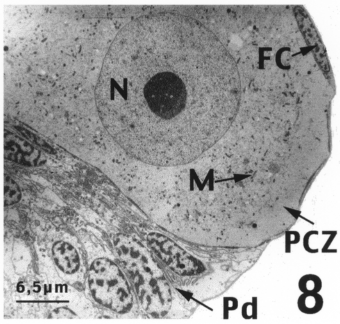
The replacement of cells occurs from undifferentiated basal cells (BC). These lie on a thick basal lamina, which is often irregular. At the basal side of the columnar cells, numerous indentations promote cohesion without obvious junctions.

Female germ cells are incorporated in the wall of ovariuterus and are noticeable by their round nuclei and clear cytoplasm, thus appear-









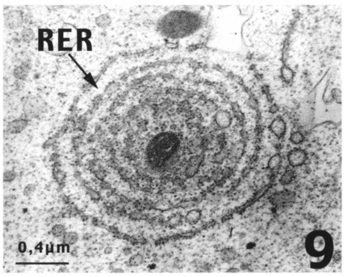


Fig. 5. Germ cell included within the epithelium.
Fig. 6. Polyedric cells of the peduncle.
Fig. 7. Semi-thin section of the peduncle. IZ: Zone for Implantation; SZ: Zone for Support; Oo: Oocyte; Ovu: Ovariuterus.
Fig. 8. Previtellogenesis stage of the oocyte. First stage. FC: Follicular Cell; M: Mitochondria; N: Nucleus; PCZ: Peripheral Clear Zone; Pd: Peduncle.
Fig. 9. Concentric Rough Endoplasmic Reticulum: RER.

ing very different from the columnar cells (Fig. 5).

The peduncle

The cells of the peduncle undergo a specific differentiation from ovariuterine columnar cells. These cells alter their direction and become polyedric, showing an important change in their nucleus structure: the nuclei become round and take on a highly granular appearance (Fig. 6). These modifications may indicate an increased activity of the cells.

The cells nearest to the oocyte infold their membrane and become filled with dense inclusions (local synthesis in these cells with transfer to oocyte?).

The peduncle differentiates later in two different parts: a zone for implantation (IZ) and a zone for support (SZ), which may have a nutritive function (Fig. 7).

The oocytes

Four stages of oocyte development are observed during oogenesis.

First stage of previtellogenesis. All the oocytes, with a size less than 100 µm, show a granular cytoplasm with many ribosomes. Two parts are

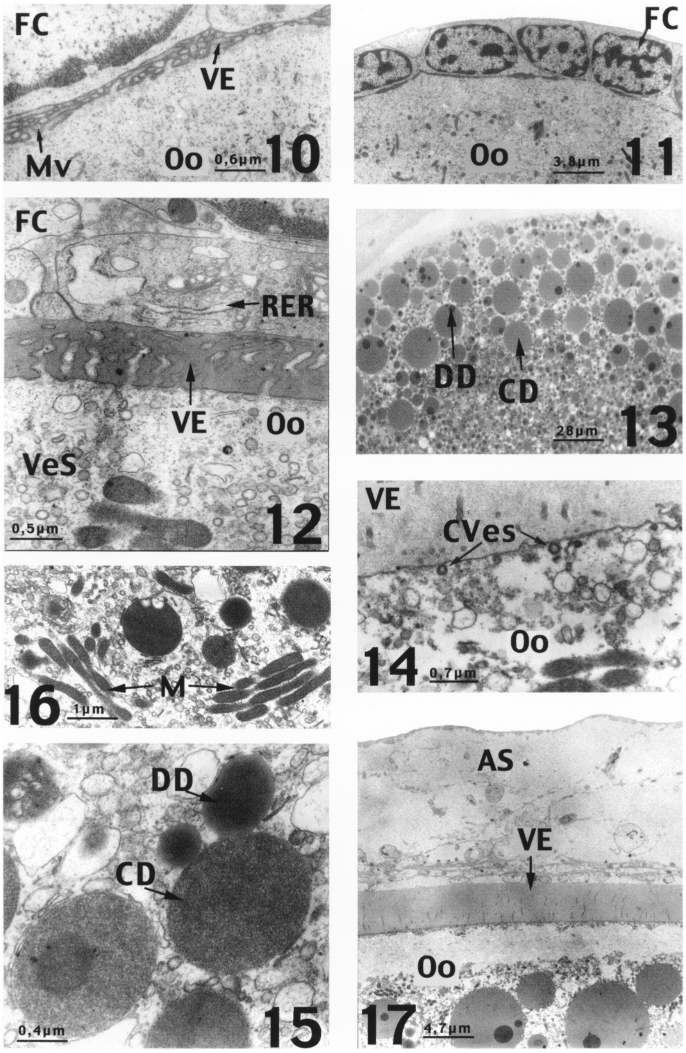


Fig. 10. Vitelline Envelope (VE). FC: Follicular cell; Mv: Microvilli; Oo: Oocyte.
Fig. 11. Cubic Follicular Cells (FC). Oo: Oocyte.
Fig. 12. Vesicular System (VeS) during the second stage of previtellogenesis. FC: Follicular cell; Oo: oocyte; RER: Rough endoplasmic reticulum; VE: Vitelline Envelope.
Fig. 13. Semi-thin section of the oocyte during the primary vitellogenesis stage. DD: Dark-staining Droplets; CD: Clear staining Droplets.
Fig. 14. Coated vesicles (CVes) associated with zone of Vitelline Envelope (VE); Oo: Oocyte.
Fig. 15. Fused yolk droplets during the secondary vitellogenesis stage. CD: Clear staining Droplets; DD: Dark staining Droplets.
Fig. 16. Groups of mitochondria (M).
Fig. 17. Thickened Vitelline Envelope (VE). AS: Nearly-Amorphous Substance; Oo: Oocyte.

currently discriminative: a peripheral clear zone (PCZ, Fig. 8) without mitochondria and a central part with many mitochondria (M) and other organelles: Golgi complex, rough endoplasmic reticulum (RER) organized in concentric structures (Fig. 9).

The formation of the vitelline envelope (VE) initially discontinuous, takes place at the level of the oocyte cytoplasmic membrane. It presents microvilli which soon close (Fig. 10).

Follicular cells (FC) form a coat created by the forward migration of some peduncular cells. They are first elongated and later become cubic (Fig. 11).

Second stage of previtellogenesis. The oocytes (100 µm to 200 µm) present similar characteristics to those of the first stage: the clear zone under the cytoplasmic membrane is still present. The cytoplasmic granulations decrease with the decreasing number of ribosomes.

The vesicular system (VeS) consists of single round or curved vesicles and of large united vesicles. Some of them are homogenous dark-staining and the others contain a granular material. The concentric reticulum is no longer observed (Fig. 12).

The continuous vitelline envelope, whose thickness increases from 0.3 to 0.8 µm, shows microvilli at wide intervals in a parallel direction.

Follicular cells overlap one another and the cellular contacts between these cells are not strong, so peculiar junctions are not observed. This membranous network could allow a permeability, which may facilitate exchanges between the haemolymph and oocyte through the vitelline envelope.

The follicular cells are now in place, very numerous and have more organelles. They could be involved in the synthesis of the oocyte reserves.

Primary vitellogenesis stage. This stage is characterized by the accumulation of yolk reserves. The density and complexity of the intracytoplasmic granules allow the recognition of two steps for storage during the growth of the oocyte.

The number of ribosomes decreases, while the cytoplasmic vesicles increase (perhaps through endocytosis). This interpretation would involve an exogenous means of establishing yolk reserves.

Dark-staining droplets (DD) appear first, followed by clearer and granular droplets (CD, Fig. 13).

The vitelline envelope can reach several micrometers (about 3 µm) in thickness and associated coated vesicles (CVes) are often observed (Fig. 14).

The follicular coat breaks up and at last we observe few fragments of cells with few organelles. Sometimes, single nuclei remain in a nearly amorphous substance. A thick basal lamina surrounds this substance.

Secondary vitellogenesis stage. The oocyte reaches its largest size and takes on an ovoid form (360 µm x 270 µm). The end of growth is characterized by the fusion of yolk droplets (Fig. 15). The more strongly-staining ones, of small size, coalesce with the clearer ones for organizing reserves into heterogeneous droplets (about 2 µm in diameter). They then form vitelline platelets.

Rare mitochondria are set side by side in characteristic groups (Fig. 16).

The vitelline envelope (VE), is very thick (about 7 µm) and no longer shows microvilli at this final stage (Fig. 17).

The follicular coat is entirely changed at the end of oogenesis, with a great development of the nearly amorphous substance (AS) of 8 to 16 µm and forms a protective layer around the oocyte. Together they protrude into the haemolymphatic cavity of the scorpion.

DISCUSSION

This is the first time that an ultrastructural study of the ovariuterus and of oocyte maturation has been carried out in *Euscorpius carpathicus*.

The germ cells are localized in the wall of the ovariuterus. But the question of whether they are able to divide or not as in Buthidae (Warburg et al. 1992, 1995) remains unanswered.

During oogenesis we can characterize 2 stages: previtellogenesis and vitellogenesis. From cytological observations, these stages are subdivided into 4 steps. During previtellogenesis, we observe many ribosomes, the Golgi apparatus, the development of a vitelline envelope around the oocyte and the follicular coat. During vitellogenesis, we observe numerous microvilli at the level of the vitelline envelope and the accumulation of yolk. This cycle is very similar to that observed in Crustacean oocytes (*Orchestia gammarella*) (Zerbib 1975; Charniaux-Cotton 1980). However, a few differences should be noted such as the membrane modification, with macrovilli that bear microvilli, and the formation of microtubules in the Crustacean.

The cytological arguments, ribosomes and RER, on the one hand, and pinocytosis, on the other hand, seem to indicate two origins: exogenous and endogenous of the oocyte reserves. If this hypothesis is correct it will be necessary to corroborate it by biochemical tests.

CONCLUSION

The development of the oocyte is similar in its main stages to that of Crustaceans (previtellogenesis, vitellogenesis). The existence of follicular cells, which are formed from ovariuterus taking place around the ovocyte, and then beginning to degenerate, is proved. Contrary to all other Arachnid groups (Kaufman 1999) the oocyte of this scorpion is surrounded by follicular cells. Our results corroborate those of Laurie (1890), who also observed follicular cells .

The peduncle, which is also formed from the ovariuterine cells, undergoes important structural modifications before ovulation.

This study will be continued by investigating the changes which occur during later development, and obviousness of vitellogenins and their incorporation.

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