Tissue and Cell 47 (2015) 456–464

Contents lists available at ScienceDirect

Tissue and Cell

journal homepage: www.elsevier.com/locate/tice

Testis follicles ultrastructure of three species of terrestrial isopods (Crustacea, Isopoda Oniscidea)

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ARTICLE INFO

Article history:
Received 25 May 2015
Received in revised form 20 July 2015
Accepted 29 July 2015
Available online 4 August 2015

Keywords:
Sertoli cell
Ultrastructure
Spermatogenesis
Phagocytosis
Spermatophore

ABSTRACT

The aim of the research, carried out on three species of terrestrial isopods – *Armadillidium granulatum*, *Halophiloscia hirsuta* and *Trichoniscus alexandrae* – is to bring a first consistent contribution to the knowledge of the ultrastructural organization of the testis follicles.

The testis follicles are seat of a remarkable dynamic activity of their cell components (somatic cells and germ cells) that results in a continuous variation, related to the trend of spermatogenesis, of their morphology, organization and of the relationships between the two cell populations.

The somatic cells, known in literature as follicular cells, nurse cells or Sertoli cells, are arranged at the periphery of the follicle to form an epithelial layer of variable thickness resting on a thin basal lamina in turn surrounded by a discontinuous network of muscle cells. In *A. granulatum* and *H. hirsuta*, two types of Sertoli cells are present: a first type, the nurse cells, envelop the spermatids in cavities within their cytoplasm and through their secretion activity play a fundamental role in the formation of the spermatophores; moreover, they phagocytizes the residual cytoplasm of spermatids. A second type of Sertoli cells shows features that leave clearly identify its supporting role to the spermatophores in formation.

In *T. alexandrae*, instead, only one type of Sertoli cells, the nurse cell, is present, whose features are widely superimposable to those observed in the other two species.

Moreover, two septa of Sertoli cells depart from the periphery of the testis follicle to constitute an articulated compartmentalization of the follicle itself, probably targeted to realize at its inside a series of microenvironments functionally diversified in order to meets the needs of the different stages of the spermatogenic cycle.

1. Introduction

The knowledge of the morphological and functional organization of the testis follicles of the terrestrial isopods is based on few outdated researches, mostly resulting from light microscopy investigations and which have sometimes provided conflicting results (Radu, 1931; Becker and Mann, 1938; Tuzet and Bessiere, 1951; Legrand, 1955; Vitagliano Tadini and Solima, 1955; Fain-Maurel, 1966; Radu and Craciun, 1969).

Radu and Craciun (1969), in particular, highlighted a significant dynamism in the organization of the testis follicles, closely correlated with the spermatogenetic process and with the spermatophore formation, and characterized by a succession of six different phases.

As in the testis of vertebrates and of many invertebrates, the male germ cells of terrestrial isopods grow, differentiate and mature in close association with somatic cells, which, because of their function, have been generally designated as nurse, follicle, supporting or Sertoli cells (Adiyodi and Adiyodi, 1983; Hinsch, 1993a; Guraya, 1995). Between crustaceans, Sertoli cells are well known in decapods (Hinsch, 1992, 1993a,b,c; Dougherty and Sandifer, 1984; Chow et al., 1991) while the knowledge of their role in terrestrial isopods is only fragmentary and primarily concerns the production of extracellular tubules associated with the spermatophores (Reger and Fain-Maurel, 1973; Itaya, 1979). More recently, some researches carried out on *Saduria entomon* (Isopoda, Valvifera) have highlighted the presence in testis follicles of two types of Sertoli cells whose different morphology and function are connected with the arrangement of germ cells in the follicle and with spermatogenesis and formation of spermatophore (Hryniewiecka-Szyfter et al., 1999; Gabala, 2006).

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http://dx.doi.org/10.1016/j.tice.2015.07.008
0040-8166/

In order to verify if also in the terrestrial isopods there is a similar organization of the testis follicles we have carried out an ultrastructural investigation on three species belonging to different families phylogenetically distant from each other, the results of which are the object of the present paper.

2. Materials and methods

The research was carried out on sexually mature males of *Armadillidium granulatum* Brandt, *Halophiloscia hirsuta* Verhoeff and *Trichoniscus alexandrae* Caruso. The specimens of *A. granulatum* and *H. hirsuta* were collected during some sampling carried in the reproductive season (April–June) in uncontaminated natural environments of south-eastern Sicily (Italy); those of *T. alexandrae* were collected inside the cave Molara, a karst cave located on the outskirts of Palermo (Sicily).

For the evaluation of the general morphology of the male genital system 5–10 males for each species were sacrificed by decapitation and dissected in Ringer's saline solution modified for terrestrial isopods according to Legrand (Besse, 1976). After removal, the male genital apparatus was observed and photographed with the aid of a stereo-microscope Wild M400, both fresh and after a brief fixation in 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.3.

2.1. Light and electron microscopy

For the ultrastructural study, five sexually mature males during intermoult stage, for each species, were sacrificed by decapitation and immediately immersed in Ringer's saline solution for terrestrial isopods and then the testis follicles were removed.

Fixation was carried out in 2.5% glutaraldehyde in 0.1 M Nacacodilate buffer, pH 7.3 for 4 h at room temperature; after repeated washing in the same buffer, the specimens were post fixed in 2% OsO_4, in the same buffer, for 1 h at room temperature.

The specimens were rinsed in water, dehydrated in ethanol followed by propylene oxide and embedded in Embed 812 (EMS).

For light microscopy, semi-thin sections, cut on a Ultracut Leica ultramicrotome with diamond blades, were stained with 0.5% toluidine blue in 0.1 M phosphate buffer, pH 7.3.

For transmission electron microscopy, ultra-thin sections, collected on Cu/Rh grids of 200 or 300 mesh, were stained with uranyl acetate and lead citrate (Reynolds, 1963) and examined in a Philips CM 10 electron microscope, at 60 or 80 kV.

3. Results

3.1. Morphology of male genital apparatus

The paired male gonads of *A. granulatum* show the general features already known for other species of terrestrial isopods (Becker and Mann, 1938; Fain-Maurel, 1966; Wägele, 1992); each gonad consists of three testis follicles scalarly inserted on the cephalic region of seminal vesicles (Fig. 1A). The testis follicles are spindle-shaped but their size and morphology vary depending on the stage of spermatogenesis that takes place in them. The seminal vesicles, knotty stick-shaped (Fig. 1A), are separated by an evident constriction from the vas deferens which continues in a thin ejaculatory duct which runs within the genital papilla.

The morphological features of the male gonads of *H. hirsuta* differ somewhat from those described for the previous species. The testis follicles appear noticeably tapered and the genital ways are more developed in length (Fig. 1B) so as to be folded twice inside the body; moreover, they present two constrictions that divide them into three distinct segments (Fig. 1B).

In *T. alexandrae*, the testis follicles are inserted closely near to each other on the cephalic region of the seminal vesicles that exhibit a diameter considerably more relevant than to the underlying deferens (Fig. 1C).

3.2. Follicle testis ultrastructure

Four different components contribute to the organization of the testis follicles: germ cells and gametes, somatic cells, basal lamina and muscular layer.

For the ultrastructural investigations we used testis follicles in phase 5 or 6 according to Radu and Craciun (1969) when they reach their maximum organizational complexity.

In the testis follicles in phase 5, about half apical follicle is occupied by spermatogonia (Fig. 1D) while in the basal half it is possible to observe the different stages of spermatogenesis; in testis follicles in phase 6 only few spermatogonia remain in the apical extremity of follicle, largely replaced by spermatocytes, while in the basal half the spermatogenesis is almost complete and many mature spermatophores are present.

In cross sections, the wall of the follicles is made of a layer of epithelial cells, known in literature as follicular cells, feeder cells, supporting cells or Sertoli cells.

The Sertoli cells rest on a basal lamina in turn surrounded by a thin layer of muscle cells circularly arranged around the follicle (Fig. 2A); the muscle cells are discontinuous and smaller in the apical region of the follicle, becoming gradually larger and tightly close to each other in the most basal region of follicle.

The basal lamina has a variable thickness and an aspect not always homogeneous; it shows a layer with a finely fibrillar organization, about 0.4–0.5 μm thick, tightly next to the basal surface of the Sertoli cells below which a layer from the structure much laxer is present (Fig. 2B).

In the apical region of the follicles in phase 5, the Sertoli cells are arranged peripherally to form an epithelial layer of irregular thickness (Fig. 1D) that in proximity of the cephalic end tends to shrink significantly. The nuclei of the Sertoli cells, slightly ovoid or pear-shaped, are relatively voluminous compared to the extension of the cytoplasm (Fig. 2A). In the cytoplasm, the organelles involved in biosynthetic activities, such ribosomes, endoplasmic reticulum and Golgi bodies, are scarcely present while there is a consistent presence of mitochondria, vesicles and small lamellar bodies (Fig. 2C). The Sertoli cells do not always extend processes between spermatogonia that are arranged to set up cordons with a radial pattern or clusters more or less extended (Fig. 1D). Spermatogonia have a roundish shape; their nuclei occupy most of the cell body (Fig. 2D) and are largely euchromatic. The cytoplasm of spermatogonia is rich in free ribosomes while the presence of rough endoplasmic reticulum and of Golgi bodies is scarce and the smooth endoplasmic reticulum is almost non-existent; moreover, numerous small lamellar bodies of heterogeneous density can be observed (Fig. 2D).

In the testis follicles in phase 6, where the half apical region is largely occupied by spermatocytes, the Sertoli cells become higher and irregularly disposed on two layers; furthermore, a layer of highly flattened cells with heterochromatic nuclei is present to delimit the lumen of the follicle (Fig. 2E).

In the region underlying that where the spermatogonia or spermatocytes are present, the Sertoli cells extend toward the center of the lumen obstructing it almost completely so as to isolate the spermatogonial compartment from the below one where the spermatogenesis takes place (Figs. 2F and 3A). In *A. granulatum* and *H. hirsuta*, in this latter region of the follicle it is possible to recognize the presence of two types of Sertoli cells clearly identifiable on the basis of their morphological and ultrastructural features and of the relationships established with the maturing gametes: a first type