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Research article

ULTRASTRUCTURE STUDY ON THE TESTICULAR SHEATHS OF *SPODOPTERA MAURITIA* BOISD. (LEPIDOPTERA: NOCTUIDAE)

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ABSTRACT: Ultrastructural studies have demonstrated that the larval testes are covered by a sheath consisting of two cellular layers, the outer tunica externa and inner tunica interna. The two layers are bounded by an outer basal lamina and an inner basal lamina. Tunica externa is thinner than tunica interna, contains numerous vacuoles and rough endoplasmic reticulum. The tunica interna is homogenous and contains numerous glycogen particles, lipid droplets, abundant mitochondria, numerous vacuoles, few pigment granules and centrally located nuclei with dense chromatin clusters. In the pupal testes, the membrana communis covers the tunica externa, but in larval testes sheath this layer is altogether absent. The cells of membrana communis are larger in size, with a prominent nucleus. Cytoplasm is homogenous with numerous glycogen droplets and mitochondria. Reduction in thickness of external layer is seen with sparse endoplasmic reticulum and few mitochondrial accumulations. The tunica interna contains abundant glycogen deposits, few lipid inclusion, mitochondria and network of channels filled with flocculent material. The cytoplasm is electron dense.The Tissue of tunica interna exhibited high secretory activity.
Key Words: Testicular sheath, Tunica externa, Tunica interna, membrana communis, *S. mauritia*.

INTRODUCTION

The lepidopteran testes have a covering of several layers called by different names in the literature. The available information on the testicular sheath of insects is comparatively scanty. Earlier electron microscopic study conducted in *Drosophila* [1] revealed that the testicular wall consists of an external layer of pigment cells and an internal layer of myoid cells. However, in most of the Lepidopterans, each testis consists of 4 follicles covered with a sheath of 3 membranes and an epithelial sheath. The structure of testicular membranes is similar to that of *Bombyx mori* and *Heliothis virescens* [2]. In *Anagasta kuehniella*, the testis wall is formed by an external cell layer that extends inward to enclose each chamber [3].

Many reports have been suggested by several authors indicating a nutritive function of the testicular sheaths. One of the prominent works was by [4] suggesting that testis sheath releases factors which stimulate meiosis. Further, the sheath can also provide nourishment or other conditions for survival of cysts, thus allowing meiosis to occur in germ cell differentiation. Hence, the present study was conducted to observe the morpho ultrastructural changes in the testicular sheaths of larval and pupal stages of *Spodoptera mauritia* Boisd.

MATERIALS AND METHODS

Sixth (last) instar larvae and pupae of the required stage were obtained from laboratory stock culture, reared and maintained in separate containers. Samples of testes of sixth instar larva and pupa were fixed for 1 hour in 3% gluteraldehyde fixative buffered with sodium calcodylate (pH 7.4) and post fixed for 1 hour in 1% osmium tetroxide. After dehydration through an ascending ethanol series, samples were embedded in Araldite mixture. Ultra thin sections cut on an ultramicrotome were contained with alcoholic uranyl acetate and lead citrate and examined with a 'JEOL' TEM 100 CX operating at 80 KV.

RESULTS

Examination of the larval testicular wall or sheath consists of two cellular layers; the internal, the tunica interna and the exernal, the tunica externa. The internal tunica folds inwards separating the follicles by septae made up of two layers of tunica interna. The tunica externa is unfolded and is absent along the interfollicular septa.

However, membrana communis, the external layer which covers tunica externa seen in pupal testes is altogether absent in larval testis sheath. The testicular sheaths are lined by two basal laminae; a thick membrane lies over the outer surface of the testis forming the outer basal lamina (Fig. 1). A somewhat thinner one marks the inner surface of the testicular sheaths forming the inner basal lamina. The inner basal lamina also covers the two surfaces of the interfollicular septa, also covers the inner surface of tunica interna facing the cysts containing the germ cells. Generally, tunica externa is thinner and more electron opaque than the tunica interna. The cells contain rough endoplasmic reticulum and numerous vacuoles. However, pigment granules and glycogen particles are absent. The cytoplasm of the outer layer is coarsely textured. In tunica interna, the cells are flat and contain numerous electron dense pigment granules and rough endoplasmic reticulum. Mitochondria are abundant. The cytoplasm of the inner layer is homogenous. The nuclei of the inner layer are large and egg shaped and occupies a central position within the cell chromatin clumps are seen scattered throughout the lumen of the nuclei. Mitochondria have a dense or dark matrix and possess a few flat cristae. In the cytoplasm, few tracheoles, multivesicular bodies, clusters of glycogen particles, numerous vacuoles, lipid droplets, large spherical proteinaceous bodies and few pigment granules are evident.

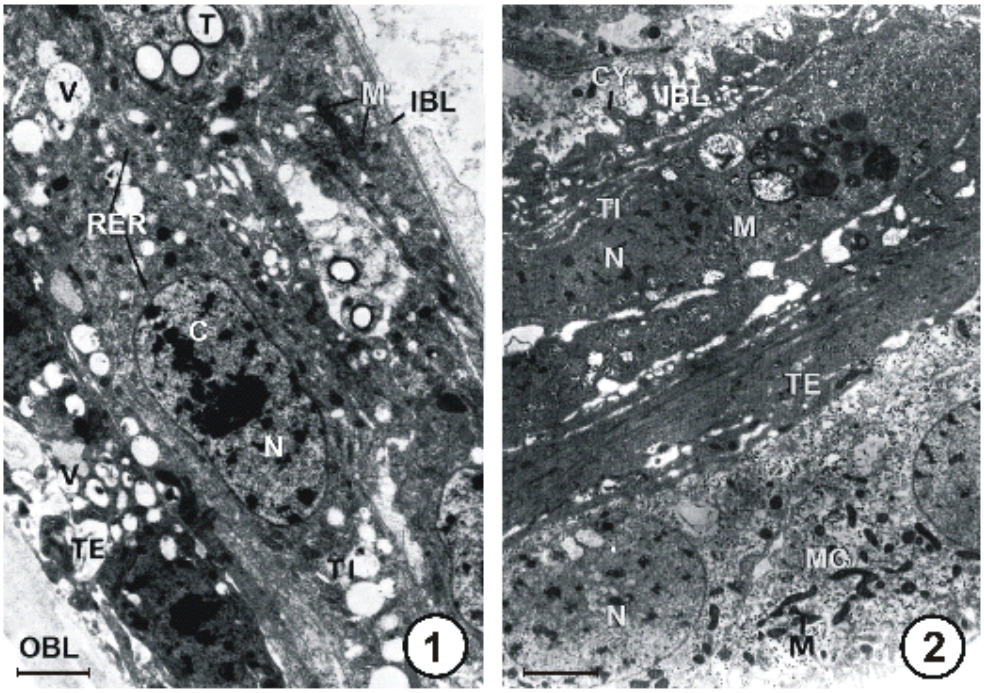

Figure.1. Testicular sheath of day 0 sixth instar larva, showing cells of tunica externa (TE) with numerous vacuoles (V) and cells of tunica interna (TI) showing a prominent nucleus (N) with chromatin clusters (C),numerous mitochondria (M),rough endoplasmic reticulum (RER),and tracheoles (T).Outer (OBL) and inner (IBL) basal lamina can be distinguished.
Bar = 2 µ m.

Figure.2. Testicular sheath of day 7 pupa showing tunica interna (TI) and membrana communis (MC),both having prominent nuclei (N).The cytoplasm contains mitochondria (M).Tunica externa (TE) lies between tunica interna and membrana communis. The inner basal lamina (IBL) shows cytoplasmic projections (CY).Bar = 2 µ m.

Ultra structure of the pupal testicular sheath

The testicular sheath of pupa is similar to that of larval testes consisting of two cellular layers, an external cell layer or tunica externa, which surrounds the testes as a whole and by an internal cell layer or tunica interna that extends inwards. The testicular sheath is lined by two basal laminae, an inner basal lamina covering the tunica interna and the outer basal lamina of tunica externa facing membrana communis (Fig. 2). The inner basal lamina made of two closely opposed layers of fibrous extracellular material, covers the tunica interna facing the cysts containing the germ cells. The inner basal lamina also covers the interfollicular septa. The inner basal laminae show numerous cytoplasmic processes or inter digitating finger like processes (Fig. 2,4).

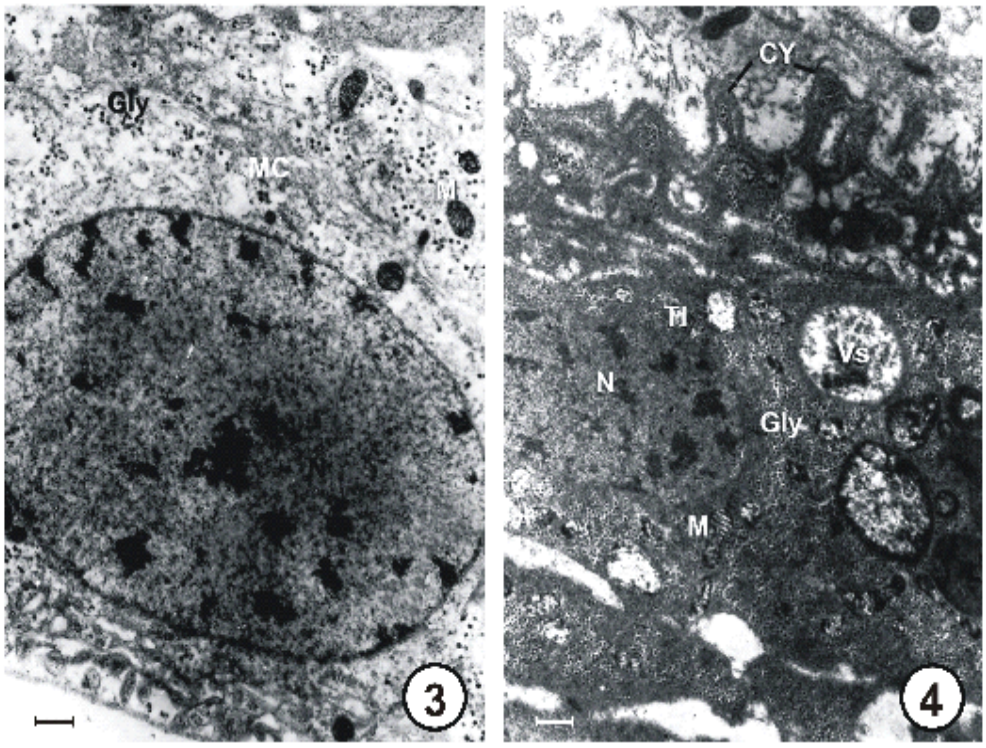

Figure.3. Section of testicular sheath of day 7 pupa showing the membrana communis layer (MC) with nucleus (N), chromatin clumps (C) and its cytoplasm containing glycogen droplets (GLY) and mitochondria (M).
Bar = 1 µ m.
Figure.4. Higher magnification of tunica interna (TI)showing nucleus (N) and cytoplasm containing mitochondria (M), numerous glycogen particles (GLY),lipid droplets (L) and vesicles (Vs).Bar =1 µ m.

The inner layer comprises numerous glycogen droplets, few lipid inclusions and vesicles. The cytoplasm is electron dense. Intercellular spaces are observed in the inner layer of the wall. These spaces constitute a network of channels filled with a flocculent material (Fig. 2, 4). Beneath the inner layer of the testis sheath lays numerous spermatid cysts and their size is smaller compared to the sheath cells. In the inner layer, the nuclei of cells are elongated and occupy a central position within cells. Clumps of chromatin are dispersed within the cell. Few mitochondria with stacks of lamellar cristae are also observed in the inner layer (Fig. 2, 4). Usually, tunica externa is thinner than the tunica interna. The thickness of the external layer is comparatively reduced in the pupal testis. In the external layer, most cells show a sparse endoplasmic reticulum and the mitochondrial accumulation are reduced to few numbers. In addition to tunica interna and tunica externa, there is another layer of testicular sheath, the membranacommunis which is present towards the exterior of tunica externa (Fig.2, 3). Membrana communis is present only in the pupal testis. Membrana communis consists of large rounded cells. The cytoplasm is somewhat homogenous with dark inclusions which probably represent pigment granules. In this layer, most cells show a sparse endoplasmic reticulum but abundant glycogen droplets and numerous mitochondria. The membrana communis is comparatively thicker than the internal and external layer. The cells observed in this layer are larger in size with a prominent nucleus. Clumps of chromatin are distributed irregularly in the nuclei (Fig. 2, 3).

DISCUSSION

The testicular sheaths of *S. mauritia* are similar in most respects to those of other lepidopteran insects, as in *Bombyx mori* [5], *Heliothis virescens* [6], *Cydia pomonella* [7], *Anagasta kuehniella* [3] and *Phragmatobia fuliginosa* [8]. The present study observed in the larval and pupal testes sheaths of *S. mauritia* only two layers are seen, an external sheath, the tunica externa and an internal one, the tunica interna. The two layers are bounded by an outer basal lamina and an inner basement lamina. In other insects, it has been reported that a single layered sheath exists in the beetle *Ipsconfusum* [9] and two continuous layers in *Drosophila melanogaster* and *Dacusoleae* [10]. In *Locusta migratoria* a discontinuous inner layer has been reported and this is external to the basal lamina [11]. In the pupal testes of *S. mauritia* there is a layer external to tunica externa, the membrana communis.

The electron microscopic studies on the testes sheaths of *S. mauritia* indicate that the tissue of the inner layer is structurally different from that of outer layer. The cells of the inner layer contain lipid and glycogen deposits, abundant mitochondria, centrally located nuclei with dense chromatin clusters and pigment granules. The inner layer also contains large amounts of proteinaceous bodies. A network of swollen channels containing flocculent material has also been observed. Similar channels containing flocculent material have been observed in *Locusta migratoria* [11] and *Anagasta kuehniella* [3]. Utilizing horse radish peroxidase as an electron dense tracer experimental studies on both species have shown that the protein molecules freely penetrates the testes sheath and readily pass through the channels into the follicles. In these insects, the channels are normally filled with haemolymph carrying nutrients and hormones to the spermatogonia. However, in *Locustamigratoria* [11], *Schistocercagregaria* [12] and in *Anagasta kuchniella* [3] it seems that there exists a blood testes barrier in the cyst envelope.

Testes of *Heliothis virescens* and *Lymantria dispar* secrete ecdysteroids *in vitro* during mid and late last instar larval stage and mid to late developmental period of the pupal stage [13-15]. Testes of *Ostrinianubilalis* were also found to synthesize ecdysteroids *in vitro* especially during late last larval instar [16]. It has also been suggested that exogenous ecdysteroids are needed to initiate [15; 7] or boost [17] endogenous ecdysteroids production of the testes *in vitro*. Immunocytochemical studies have indicated that the inner layer of testicular sheath synthesize and release ecdysteroids [18]. However, this study does not rule out the possibility that the immunoreactive steroids observed in the testes sheath cells were sequestered from haemolymph or from the lumen of the testes into the inner layer of the sheath for later processing and/or release [18]. Transmission electron micrographs of the testicular sheaths of *S. mauriti a*s how that tissue of tunica interna exhibits high secretory activity. The tissue of inner layer is structurally different from that of the outer layer and contains glycogen deposits, abundant mitochondria and a peculiar network of swollen channels containing flocculent material. These features suggest that the tissue contain steroid secreting cells. However, the presence of rough endoplasmic reticulum and large proteinaceous bodies do indicate that these cells might be involved in the synthesis of proteins which might have a critical regulatory role in spermtogenesis. In fact, there is a large body of evidence in literature that testes sheaths synthesizes certain proteins and liberates them into follicles and testicular lumen. These proteins promote spermatogenesis in *Mamestra brassicae* [19]. In *Heliothis virescens* and *Lymantira dispar* testes sheath and fat body of developing male pupae synthesize soluble growth factor like substances when exposed to ecdysteroids. These growth factors are needed for meiosis to occur in spermatocytes cultured *in vitro*. As growth factors couple to their receptors, the gene activation and transcription follows in the induction of cell division, differentiation and development [20]. However, the report of testes sheath of *Heliothis virescens* [4] release factors which stimulate meiosis in cultured spermatogonial cysts and the effects of testes sheath is dose dependent and varied with donor's age. In conclusion, the ultra structural investigation on testicular sheath of paddy pest, *Spodoptera mauritia* marked structural differentiation into two cellular layers, inner and external, of which the inner one exhibited high secretory activity.

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