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Testicular Reabsorption in Adult Males of *Melipona bicolor bicolor* Lepeletier (Hymenoptera, Apidae, Meliponini)

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Summary The present paper reports morphological features of the testes reabsorption in adult male of *Melipona bicolor bicolor.* The ultrastructural features of the degenerating sperm cells and cyst cells are described. It is suggested that the material of the reabsorbed testes may serve as nutritional source for the adult male.

Key words Testes, Reabsorption, Males, Sperm cells, Cyst cells.

Several reports give account of cellular reabsorption during spermatogenesis in insects, under physiological or patological conditions. The reabsorption may occur at any developmental stage of the spermatogenesis, although less frequently in spermatogonia.

A small rate of cell reabsorption always occurs, serving to discarding the damaged cells originated from random errors during cell proliferation or meiosis (Beaulaton and Perrin-Waldemar 1973, Perrin-Waldemar and Beaulaton 1973) and is considered physiological. More elevated rates of reabsorption may be due to inadequated environmental conditions, desease or intoxication (Szollosi 1976a, b).

In bees as in several other holometabolous insects, the spermatogenesis occurs in the imature, generally during pupation. When the adult emerge it is finished and the spermatozoa abandon the testis to the seminal vesicle (Chapman 1998). Cruz-Landim *et al.* (1980) reported sperm cell reabsorption in bees during the early spermatogenesis and in already differentiated spermatozoa, still inside of pre-pupae seminiferous tubules of *Apis mellifera*, *Scaptotrigona postica* and *Melipona quadrifasciata anthidioides.*

The present report deals with the testis degeneration in the newly emerged adult of *Melipona bicolor bicolor.*

Material and methods

Testis of newly emerged and mature males, captured in colonies of *Melipona bicolor bicolor* raised in the Instituto de Biociências de Rio Claro (UNESP), SP, Brazil, apiary, were fixed for transmission electron microscopy (TEM) in 2.5% glutaraldehyde in 0.1 M Na Cacodylate buffer, pH 7,4. The testes after washed in the buffer were pos-fixed in 1% osmium tetroxide in same buffer. The pieces were then dehydrated in a series of increasing concentration of acetone. During the beginning of the dehydration the pieces were stained with 2% uranyl acetate in acetone. The embedding was done in Epon Araldite and the thin sections were stained with lead citrate before being examined and photographed at TEM. Semi-thin sections were stained with 1% toluidine blue and used for light microscopy (LM) examination.

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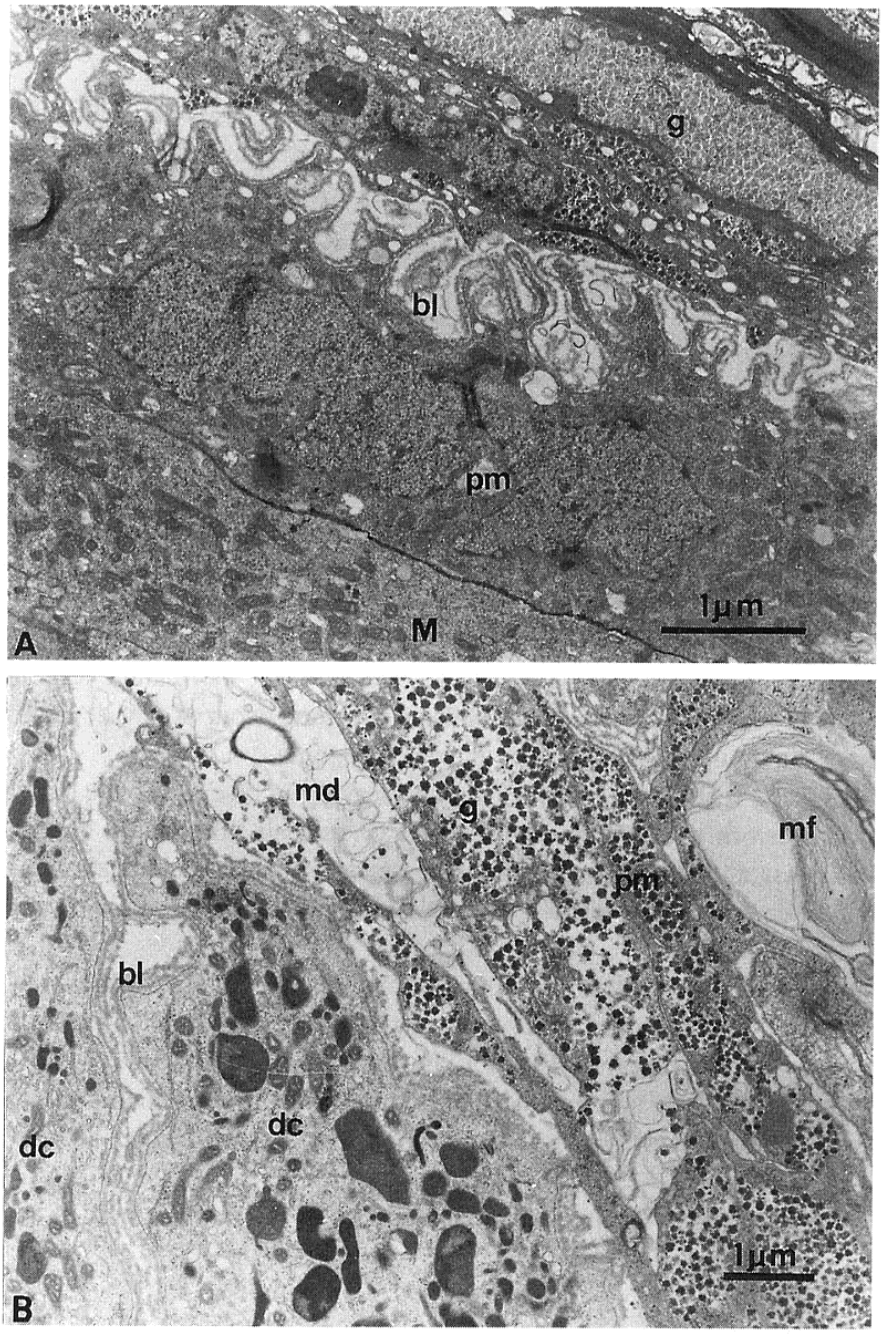

Fig. 1. A and B. TEM micrographs of the outer peritoneal membrane (pm) of adult testes of *Melipona bicolor bicolor*, showing glycogen (g) and the pleaded basal lamina (bl). M=muscle, dc=degenerative cyst, mf=myelin figure, md=membranous debris.

Results

In the newly emerged males, the testis appear as a white mass of tissue colapsed over the seminal vesicles. In this mass their anatomy are not recognized anymore. In mature males, this mass have disappeared almost completely.

In sections of the testicular mass of the newly emerged adults, can be distinguished some tubules with their peritoneal sheath. These tubules are colapsed and the basement membrane of the peritoneal sheath pleated (Fig. 1A). The enveloping cells show great amounts of glycogen (Fig. 1A, B) and between them there are several membranous debris that may be arranged as myelin figures (Fig. 1B).

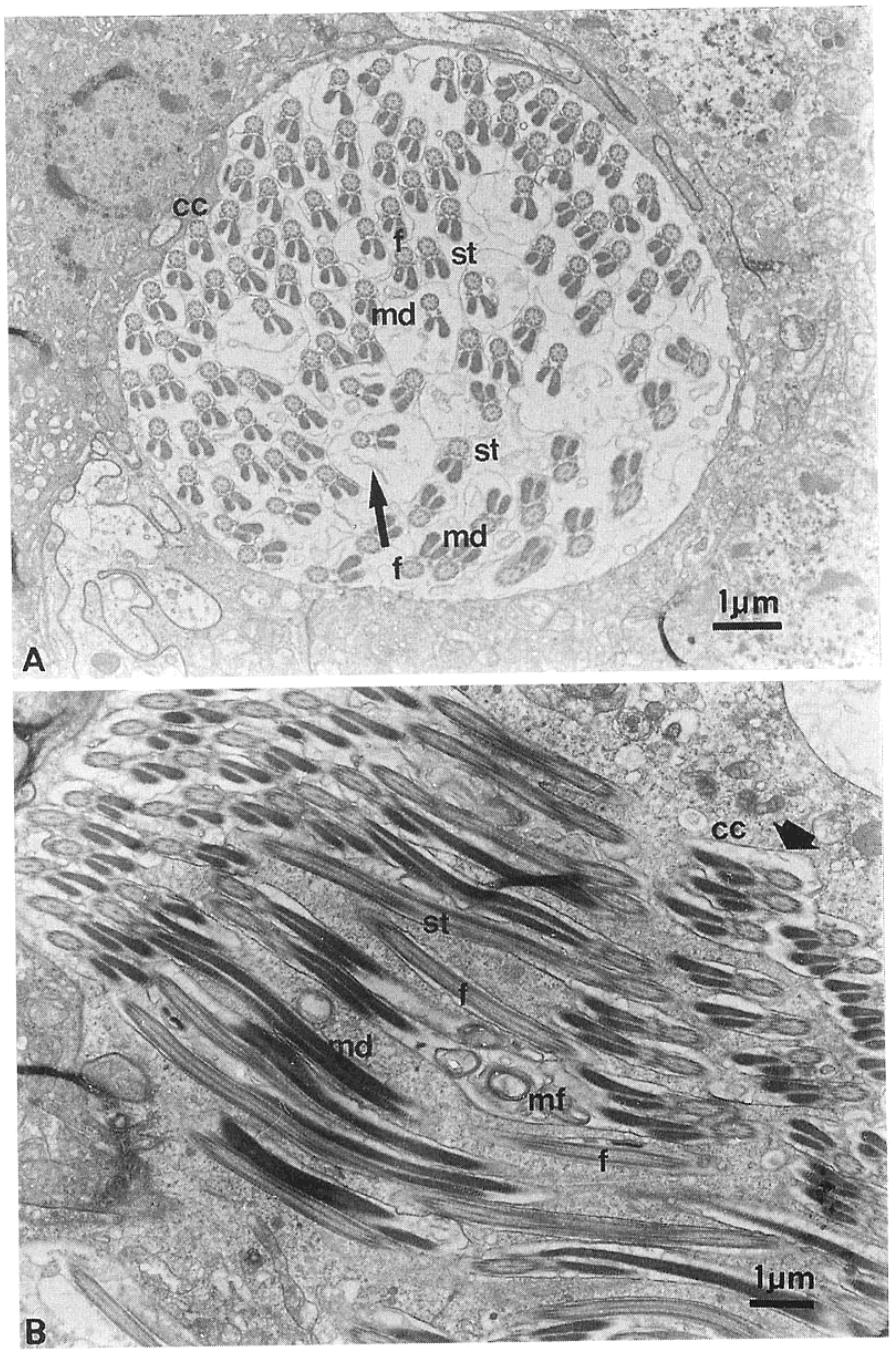

Fig. 2. Cross section (A) and Lengthwise section (B) of almost intact sperm tails (st) inside the testicular cysts. Notice the swelling of the plasmic membrane in A (arrow) and the tail within vacuoles of the cyst cell (cc) in B (arrow). dm=mitochondrial derivative, f=flagella.

In the tubules that are still preserved, it is possible to observe cysts containing sections of spermatozoa. The sections passing through the sperm tail, show almost intact features in some cysts and disorganized tails in others (Fig. 2A, B). The cyst cells seem to have increased in size, presenting larger cytoplasm then can be seen in the functional testis. But even the cysts that seem to be intact, present some disorganization of the sperm tail (Fig. 2A, B). While the flagella and mitochondrial derivatives seem inaltered, the tail plasmic membrane appear swelled, forming an apparently empty and large space around these structures (Fig. 2A). Membranous debris, sometimes with concentric arrays are seen among the sperm (Fig. 2A, B).

The flagella and mitochondrial derivatives remain morphologically integrous even in badly disarranged tails (Fig. 3A). The disruption of the tail is characterized by the appearance of piles of

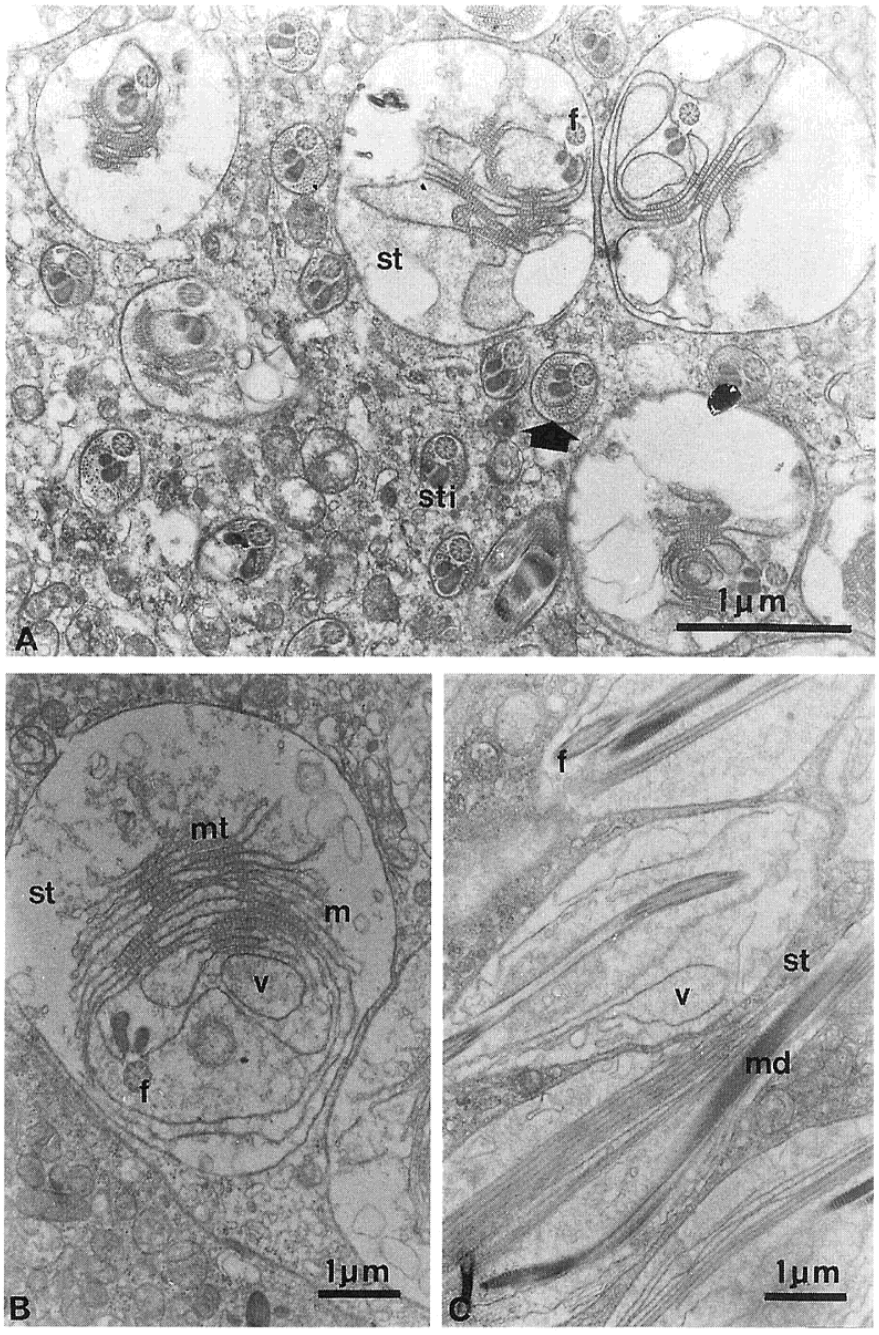

Fig. 3. TEM micrographs of degenerating sperm tails (st). A) Intact (sti) and degenerating (st) sperm tails. The arrow point to microtubules in the intact sperm tails. B) Cross section of degenerating sperm tail (st), showing the flagellum (f) and the mitochondrial derivatives (dm). The arrows point the continuity of the membranes (m) with the mitochondrial derivative. C) Lengthwise section of the analogous sperm tails (st). v=vacuoles, mt=microtubules.

smooth membranes separated by rows of microtubules (Fig. 3A, B, C). The microtubules are arranged parallel to the tail lenght (Fig. 3C) and seem to be originated from microtubes present in the intact tails (Fig. 3A). However, the origin of the membranes were not detected, although they seem to be continuous with the outer membrane of the mitochondrial derivatives (Fig. 3B). These membranes delimitate inner spaces, mostly closed, but with small dilatations in the borders, and sometimes enlarged to produce vacuoles, filled with floculent material (Fig. 3A, B). These disintegrating spermatozoa seem to be located inside the vacuoles of the cyst cell (Figs. 2A, 3A, B, C).

In more advanced reabsorption process, the flagellum and the mitochondrial derivative appear

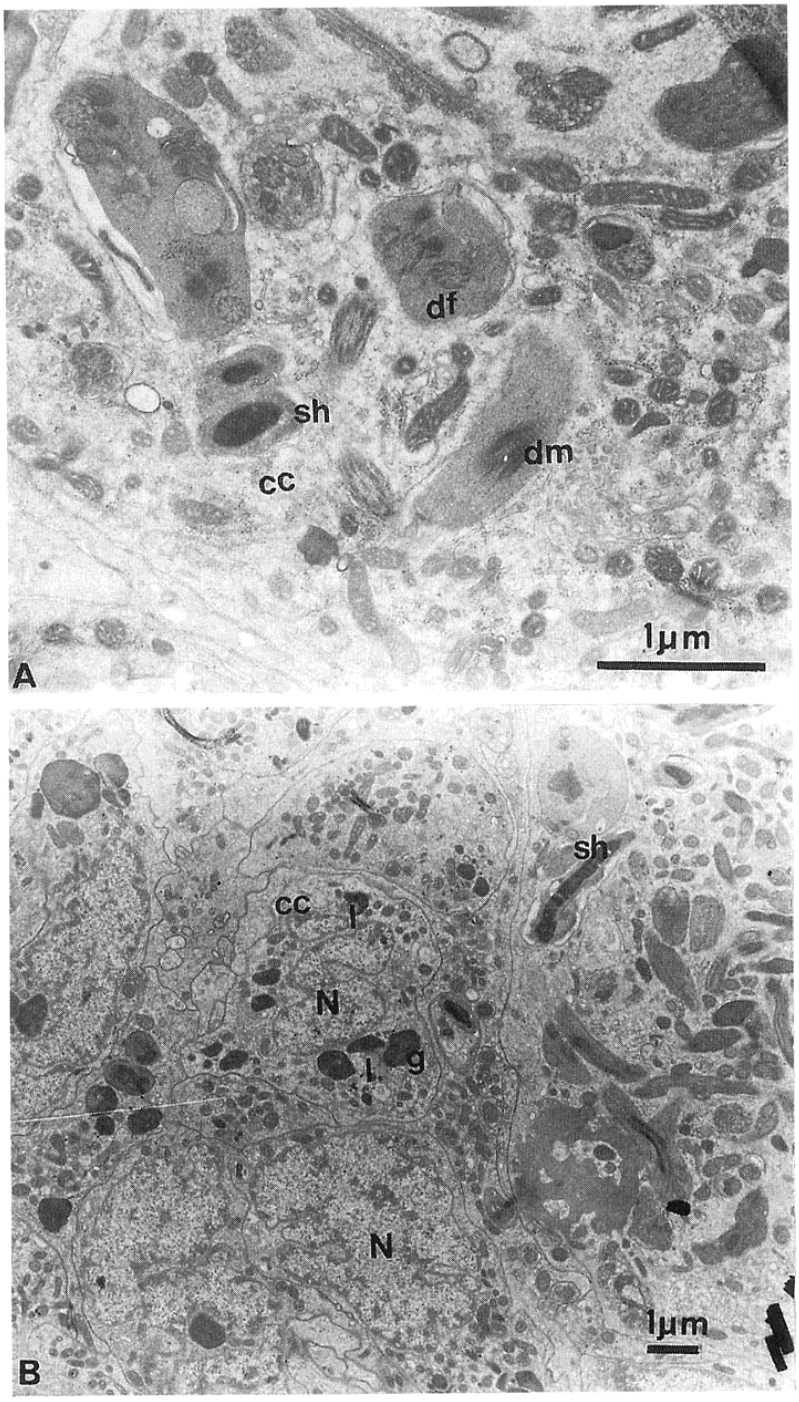

Fig. 4. TEM of the advanced reabsorption of the spermatozoa, showing in A and B disrupted flagellum (df) and the mitochondrial derivatives (md) into the cyst cells (cc). Notice in B a possible cyst cell (cc) containing electrondense granules (g) and lipid droplets (l). sh=sperm head, N=nucleus.

disrupted, and now immerse in an amorphous material into vacuoles of the cyst cell. Sometimes membranous structures are also present into these vacuoles (Fig. 4A).

The nuclear region of the sperm cell was rarely observed in sections of the degenerating testis. However some high electrondense structures present in more advanced rebsorption phases were interpreted as representing the sperm head (Fig. 4A, B). These electrondense structures also appear sequestered into the cavities delimited by membranes into the cyst cell cytoplasm (Fig. 4A, B).

Some large cells, with irregular nuclei and cytoplasm rich in mitochondria and electrondense granules (Fig. 4B) were interpreted as cyst cells where the remaining sperm cells have been totally

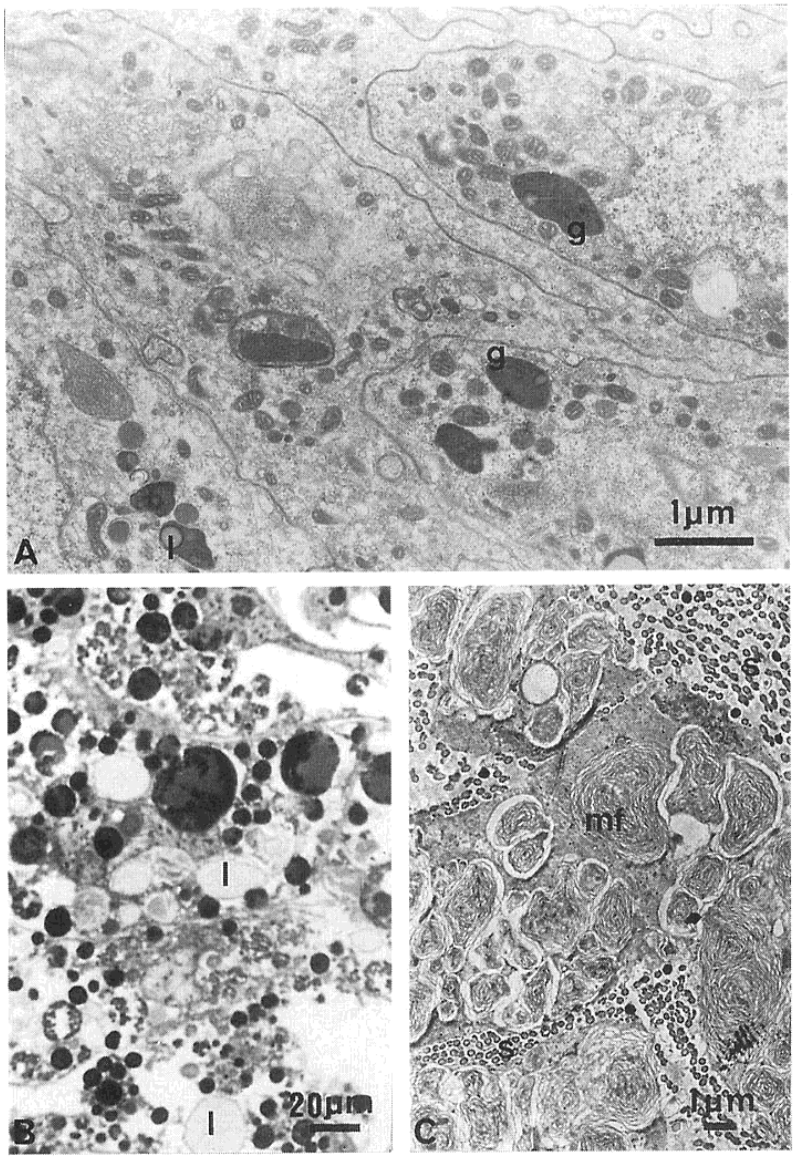

Fig. 5. TEM of the final reabsorption of the spermatozoa. A) Electrondense granules (g) and lipid droplets (l) as final result of the reabsorption. B) Light micrograph of a Toluidine blue stained section of the degenerating testes. C) Myelin figures (mf) among the spermatozoa (s) in the basal seminiferous tubule.

digested (Fig. 4B). The electrondense granules apparently tend to develop lipid droplets inside, or turn into lipid droplets (Figs. 4B, 5A). The degenerated testis appear to the light microscopy as a irregular mass of tissue containing lipid droplets and numerous granules that darkly stains by the toluidine blue (Fig. 5B).

It seems that even in the sperm that were delivered from the testis, but have not reached the seminal vesicle, is reach by the degeneration as suggested by the presence of numerous myelin figures among the spermatozoa (Fig. 5C) into the vas deferens.

Discussion

The spermatogenesis in the studied bee take place during the pupation, therefore when the adult male emerge the sperm are descending to the seminal vesicle and the testes being reabsorbed.

The reabsorption of the remaining sperm cell, after copulation in the male tract of insects is a

common occurrence (Szollosi 1976a, b), as well as in the other animals, for instance in the ones that have seasonal cycles of reproduction.

The features observed in the sperm cells that remain in the testes and in the testis cells itself seem to indicate that the cyst cells have a preponderant role in the remaining sperm digestion.

Also, the morphological features indicate that the final products of the reabsorption may be, in some case, lipids as suggested by the great amount of membranous debris, myelin figures and lipid droplets.

The remaining of the testes, in some way take the aspect of the fat body, the restant cells are filled with glycogen, lipid and granules that can be proteic. This fate of the testes, may suggest that the males use this material as source for nutritive substances in addition to the food intake during adult life.

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