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HISTOLOGICAL AND ULTRASTRUCTURAL ASPECTS OF THE FAT BODY IN VIRGIN AND PHYSOGASTRIC QUEENS OF *Melipona quadrifasciata anthidioides* LEPELETIER, 1836 (HYMENOPTERA, APIDAE, MELIPONINI)*

Vagner Tadeu Paes-de-Oliveira and Carminda da Cruz-Landim

Department of Biology, Institute of Biosciences, Paulista State University (UNESP), Rio Claro, SP, Brazil

ABSTRACT

Variations in the morphology and biochemical content of insect fat body have been associated with metabolic activity and the reproductive cycle (synthesis of vitellogenin). In social insects such as bees, the functional traits of fat body also differ between workers and queens. In this work, we used light and transmission electron microscopy to examine the morphological features of fat body trophocytes of virgin and physogastric mated queens of the stingless bee *Melipona quadriafasciata anthidioides* before and during vitellogenesis. Virgin queens had few, small fat body cells in which lipid deposits predominate, and showed no evidence of biosynthetic activity or the uptake of exogenous substances. In contrast, the fat body cells of physogastric queens were almost completely devoid of lipids, exhibit a well-developed rough endoplasmic reticulum with an obvious intraluminal product, and contained Golgi stacks that release numerous vesicles. These ultrastructural findings were suggestive of proteosynthesis. However, there was no evidence for the accumulation of synthesized material in the form of secretory granules. We conclude that the trophocytes of virgin and physogastric queens differ basically in their switch from a storage role in the former to a synthetic role in the latter. In addition, the high level of vitellogenesis seen in egg-laying queens suggests that the main material synthesized is vitellogenin.

Key words: Ovarian development, queens, trophocytes, ultrastructure, vitellogenesis

INTRODUCTION

In insects, the fat body has a mesodermal origin and is associated primarily with intermediate metabolism [23], although production of the specific female protein vitellogenin has also been attributed to fat body [18]. Fat body is located in the perivisceral and parietal compartments of the abdomen, as well as in the thorax, head and even the appendices**.** Fat body cells always appear to be in contact with the hemolymph, a condition that facilitates metabolic exchange with the circulating medium.

The shape and other aspects of trophocytes, the main cell type representative of fat body tissue, vary depending on the developmental and nutritional state of the insect [23]. The lipid deposits generally occupy most of the cytoplasm, with the remaining space being occupied by the nucleus and other organelles such as the rough endoplasmic reticulum, mitochondria, Golgi complex and lysosomes [4].

The occurrence of a peak in protein production by females fat body has been associated with the reproductive cycle and coincides with yolk deposition in the oocyte [1,8,16,30]. In social insects, reproductive females have larger fat body than workers. Virtually only parietal tissue occurs in the abdomen of adult *Apis mellifera* workers, while in the queen such tissue also occurs in the visceral compartment [5]. In many higher insects, such as the Hymenoptera, the oocytes or other ovarian cells do not synthesize yolk [13,14], but nutrients are acquired by the uptake of a soluble precursor, vitellogenin, that is synthesized by the fat body and released into the hemolymph [11,31].

Morphological and biochemical changes have been reported in the fat body of several species, in agreement with the biosynthetic activity of trophocytes involvement in the production of

Correspondence to: Dr. Carminda da Cruz Landim
Departamento de Biologia, Instituto de Biociências de Rio Claro, Universidade Estadual Paulista (UNESP), CP 199, Av. 24A n.1515, Bela Vista, Rio Claro, SP, Brazil, CEP 13506-900. Tel: (55) (19) 3526-4135, Fax: (55) (19)3526-4136.
E-mail: vagnertadeu@yahoo.com.br, cclandim@rc.unesp.br

vitellogenin [12]. In *Nauphoeta cinerae*, the fat body undergoes considerable modifications during vitellogenesis, including an increase in the amount of rough endoplasmic reticulum in the trophocytes at the beginning of the reproductive cycle [2]. Approximately five days later, the cells enter a phase of high protein synthesis and accumulate large amounts of lipids and proteins that result in marked morphological transformations in the cytoplasm. However, by the end of the reproductive cycle, the synthetic complex becomes inactive and disappears [22,32]. In *Chrysomya bezziana*, the fat body is well-developed at emergence but decreases to 70% of its original size after vitellogenesis [28]. Staurengo da Cunha and Cruz-Landim [29] observed that in queens of the ant *Atta sexdens rubropilosa*, 18 days after the mating flight the ovaries grew at the expense of material stored in the fat body since the ovarian cells appeared to be devoid of reserves.

The aim of this study was to examine the morphological characteristics of trophocytes in virgin queens and to determine the modifications that occur when the mated queen becomes physogastric and there occur oocyte vitellogenesis.

MATERIAL AND METHODS

Portions of the fat body of virgin and laying queens of *Melipona quadrifasciata anthidioides*, collected from the meliponary of the Institute of Biosciences at UNESP, Rio Claro (SP), were used. Three newly emerged queens were collected as soon as they broke the capping of the brood cells and three mated queens were collected from three colonies of similar sizes when they showed physogastry and egg oviposition.

Light microscopy (LM)

For light microscopy, whole abdomens were separated from the rest of the body, fixed in 4% paraformaldehyde in 0.1 M sodium phosphate buffer (pH 7.2) and embedded in resin (JB4 or Leica). Sections 5-6 μm thick were cut and stained with hematoxylin and eosin (HE).

Transmission electron microscopy (TEM)

Fragments of fat body were fixed in a mixture containing 2% glutaraldehyde and 4% paraformaldehyde in 0.1 M sodium cacodylate buffer (pH 7.4). After washing in this buffer, the fragments were postfixed in 0.5% osmium tetroxide containing 0.8% iron-potassium cyanide in the same buffer and at the same pH as above. The material was rinsed in a 0.1% aqueous solution of tannic acid, contrasted with 2% ethanolic uranyl acetate, dehydrated in acetone (50-100%) and embedded in Epon-araldite. The ultrathin sections were counter-stained with lead citrate.

RESULTS

In virgin and physogastric queens, fat body is seen in the visceral and parietal compartments, being more abundant in the latter. The visceral compartment is located close to the basal region of the ovary and is more developed in physogastric queens. Although numerous oenocytes are seen among the trophocytes, our emphasis here will be on the trophocytes.

Virgin queens

The fat body of virgin queens has trophocytes with an acidophilic cytoplasm containing large vesicles and an irregularly shaped nucleus with several nucleoli, in addition to oenocytes (Fig. 1A). The trophocytes of the parietal compartment contain lipid and protein deposits and urate granules. The heterogeneity of some granules suggest that they are of a lysosomal origin. These cells are provided with invaginations of the plasma membrane that formed a shallow peripheral labyrinth (Fig. 2A) and a thin basal lamina. The Golgi complex is poorly developed (Fig. 2B), and there are few cisternae of the rough endoplasmic reticulum, which rarely contain electron-dense deposits in their lumen. The mitochondria varied in shape and size, and the nuclei are round and euchromatic, with few nucleoli in the central region. There are also lamellar bodies that form aggregates and granules of medium electron-density (Fig. 2C), probably resulting from autophagy.

Lipid droplets are rare in trophocytes of the visceral compartment, although granules of medium electron-density, probably containing proteins, were seen. The trophocytes had a shallow peripheral labyrinth, a thin basal lamina (Fig. 2D), round mitochondria, cisternae and a poorly developed rough endoplasmic reticulum. The nuclei are large and irregular, and contain several nucleoli. Granules of varying sizes and electron-densities are seen around the Golgi complex.

Physogastric queens

In physogastric queens, the fat body includes similar-sized trophocytes and oenocytes (Fig. 1B). The oenocytes had small, homogenous nuclei and an acidophilic cytoplasm, in contrast to the trophocytes that contain a relatively large nucleus with many nucleoli (Fig. 1D). The cytoplasm contain few lipid inclusions that appear dappled in contiguous areas after staining with HE. A narrow basophilic zone is present around the nucleus whereas a thin strip stained with HE occurrs at the cell periphery (Fig. 1C,D).

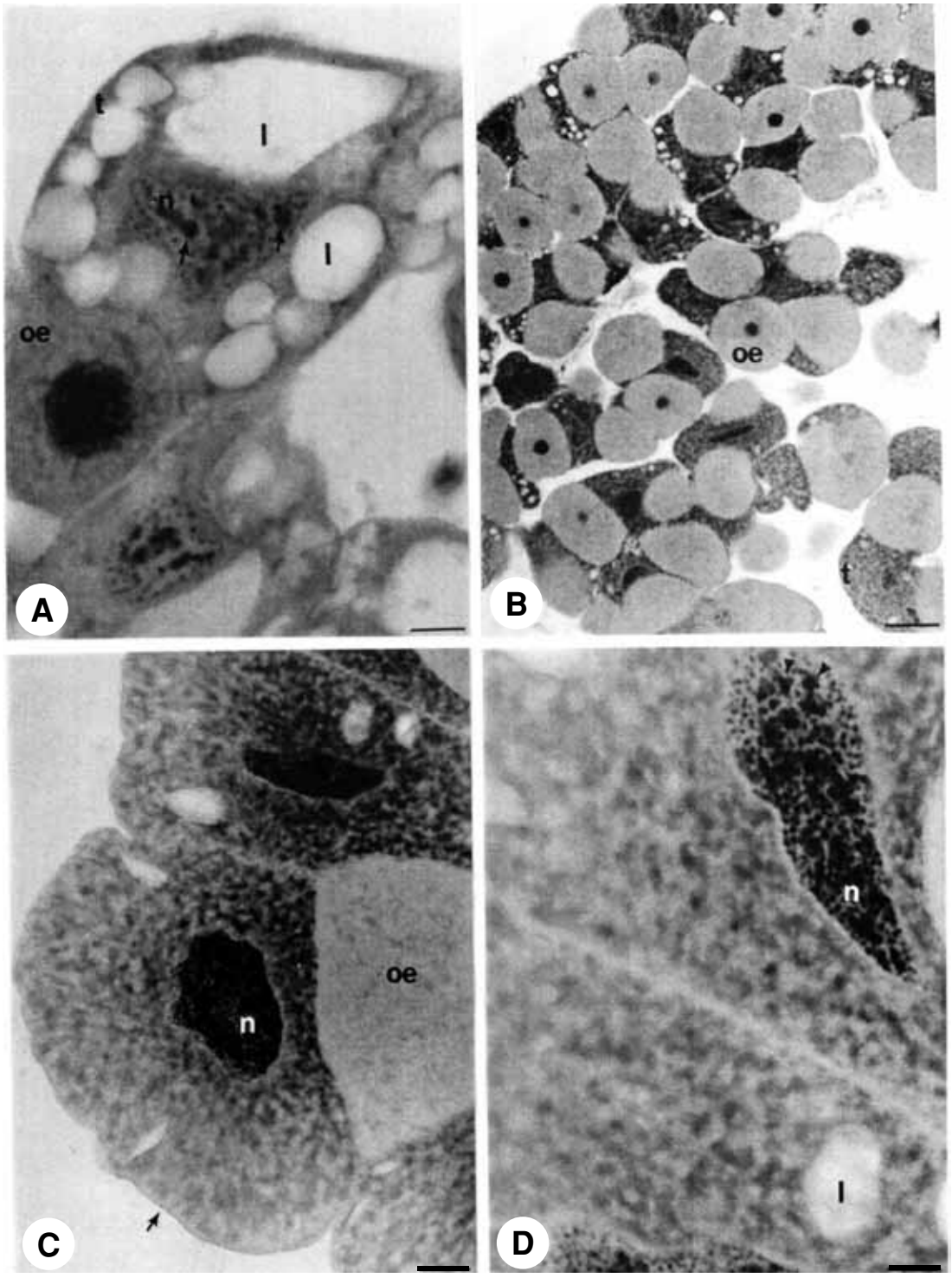


Figure 1. Histological features (LM) of parietal fat body cells in queens of *M. q. anthidioides* (HE). **A)** Trophocytes (**t**) and oenocytes (**oe**) are the two cellular types in the fat body tissue of virgin queens. Their nuclei (**n**) resemble those of physogastric queens because of their large size and numerous nucleoli (**arrows**). Bar = 15 µm. **B)** Main view of a parietal fat body in a physogastric queen showing that this organ is formed by trophocytes (**t**) and oenocytes (**oe**). Bar = 60 µm. **C)** Trophocyte (**t**) of a visceral fat body in a physogastric queen showing its granular cytoplasm. The **arrow** indicates a basophilic cell. The cytoplasm of the oenocytes (**oe**) is translucent and homogenous. Bar = 15 µm. **D)** Detail of a visceral fat body trophocyte in a physogastric queen showing the large nuclei (**n**) with numerous nucleoli. Note that lipid droplets (**l**) are rare in these cells. Bar = 6 µm.

The ultrastructure of trophocytes from the parietal and visceral fat body of physogastric queens differs from that of virgin queens. The cisternae of the rough endoplasmic reticulum form clusters that resembled spirals and are separated by areas where the other cell organelles are located (Fig. 3A). The cisternal lumen is filled with material of intermediate electron-density. In the Golgi region, the endoplasmic reticulum consists of smooth profiles (Fig. 3E). The lipid droplets are small, few and disperse, and the mitochondria are ribbon-like and elongated. Vesicles of different sizes and electron-densities are present in the cytoplasm (Fig. 3B-D,F). Electron-dense deposits are also present in vesicular compartments of the endoplasmic reticulum (Fig. 3F), and lamellar structures are seen surrounding protein granules and mitochondria in other areas of the cytoplasm, indicating autophagic processes. The occurrence of heterogenous, irregularly shaped granules also suggest the occurrence of autophagy.

DISCUSSION

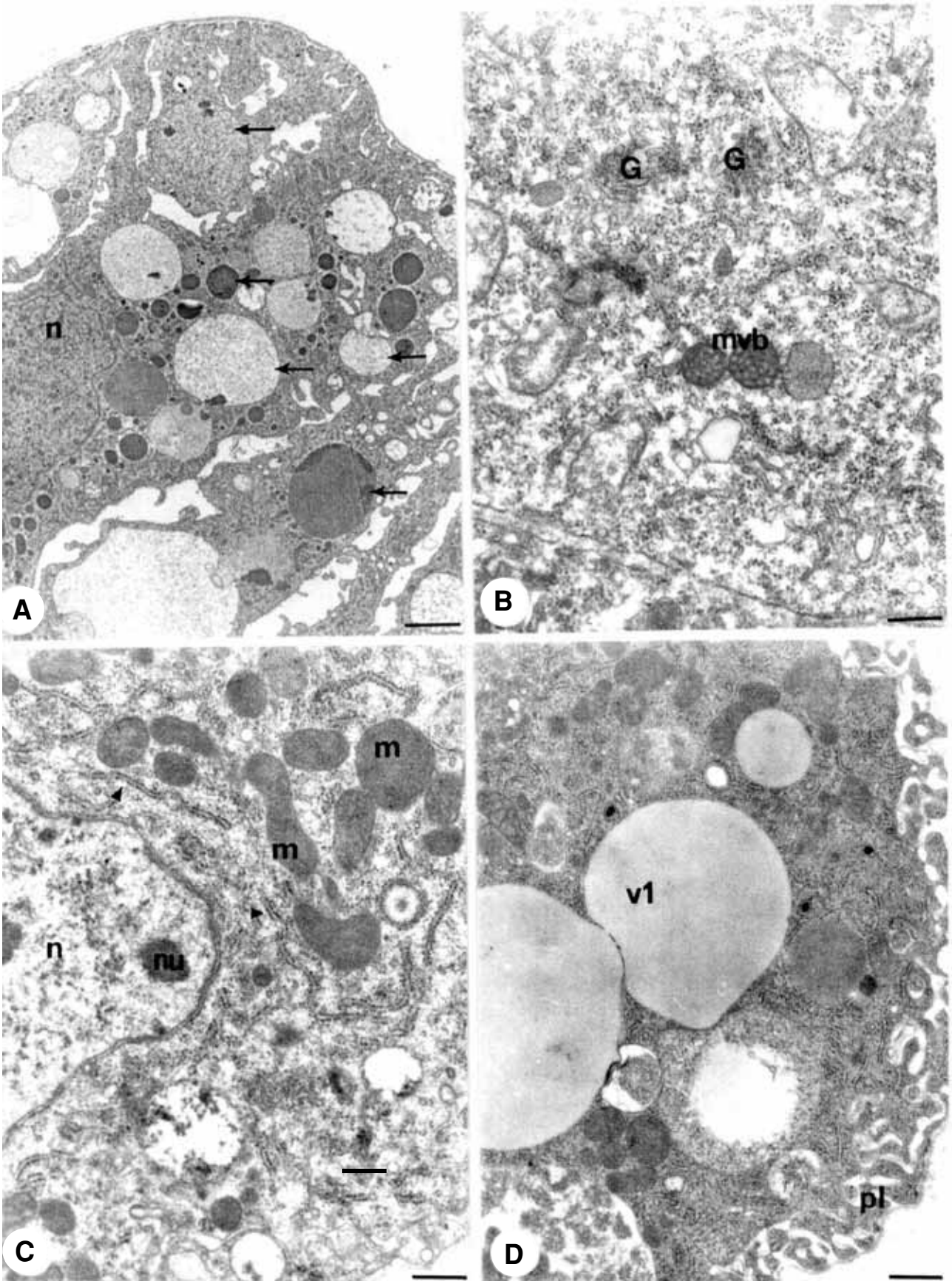
In virgin queens of *A. mellifera* [27] and *M. quadrifasciata* [7,25], the ovaries are still in the pre-vitellogenic phase so that the small size of the trophocytes probably reflects the undeveloped state of the ovary. In *A. mellifera* queens, vitellogenin is produced at a high concentration in the fat body from the fourth day of adult life onwards [17], although there is evidence that vitellogenesis may not start before fertilization [27]. The vitellogenin produced in the initial stage of queen development is released into hemolymph where its concentration regulates the synthesis of this molecule by fat body [11]. In *A. mellifera*, vitellogenin is absorbed through the ovarian follicular epithelium to be provided to oocytes [9,10]. Vitellogenin levels in the hemolymph are regulated by a balance between the rate of synthesis in fat body trophocytes and uptake by the ovary [3,14].

The trophocytes of virgin queens are smaller than those of physogastric queens [26]. In newly emerged queens of bee species with trophic caste determination, the fat body is more developed than in newly emerged workers because the queens eat more and have a larger fat body during larval life [5]. This is not the case in the species studied here, which has genetically determined castes [19] and the virgin queen emerge with poorly developed fat body. The laying queen is fed with trophic eggs laid by the workers and the fat body grows at the expense of worker eggs consumption. As shown here, despite their small size, the trophocytes of virgin queens contained large lipid deposits that occupy almost the entire cytoplasm, a characteristic that differentiat them from the trophocytes of physogastric queens. However, their nuclei resembled those of physogastric queens, i.e., they are large and irregular, with many nucleoli.

The trophocytes of the fat body in physogastric queens are nearly five times larger than the corresponding cells of virgin queens. This greater cell size is apparently unrelated to the amount of substances stored intracellularly since this material (mainly lipids) decreased compared to the trophocytes of virgin queens, but rather represent the growth of rough endoplasmic reticulum, as shown by light microscopy and transmission electron microscopy. There are virtually no large vesicles or cytoplasmic granules in the trophocytes of physogastric queens. The ultrastructural analysis revealed a small number of granules and vesicles, with little diversity among them. The accumulation of electron-dense material, possibly iron [6,21], has been observed in these cells. These deposits may represent the neutralization of the toxic effects of iron, which is sequestered by specialized cells in some organisms [24], rather than being used for magnetic orientation [15,20].

The cytoplasm of fat body trophocytes is characterized by basophilic islands, an outstanding trait of protein synthesis. The rough endoplasmic reticulum is highly developed and formed by spiraled clusters of cisternae in which the lumen is filled with material of medium density. In addition to the Golgi apparatus, the presence of pseudomyelinic structures indicates the intense turn-over of patches of internal and plasma membranes, as also indicated

Figure 2. TEM of the fat body in virgin queens of *M. q. anthidioides*. **A)** Main view showing several types of granules and vesicles (**arrows**) in the parietal compartment. The plasma membrane is detached from its basal lamina and forms a shallow peripheral labyrinth. **n** = nucleus. Bar = 2 μm. **B)** Detail of the cytoplasm of a parietal fat body trophocyte, showing the Golgi apparatus (**G**) and multivesicular bodies (**mvb**). Bar = 0.8 μm. **C)** Fat body trophocyte of the visceral compartment showing rough endoplasmic reticulum profiles (**arrowheads**) along with many mitochondria (**m**); **n** = nucleus, **nu** = nucleolus. Bar = 1 μm. **D)** Trophocyte of the same compartment showing peripheral labyrinths (**pl**), vesicles (**v1**) and a poorly developed rough endoplasmic reticulum. Bar = 0.8 μm.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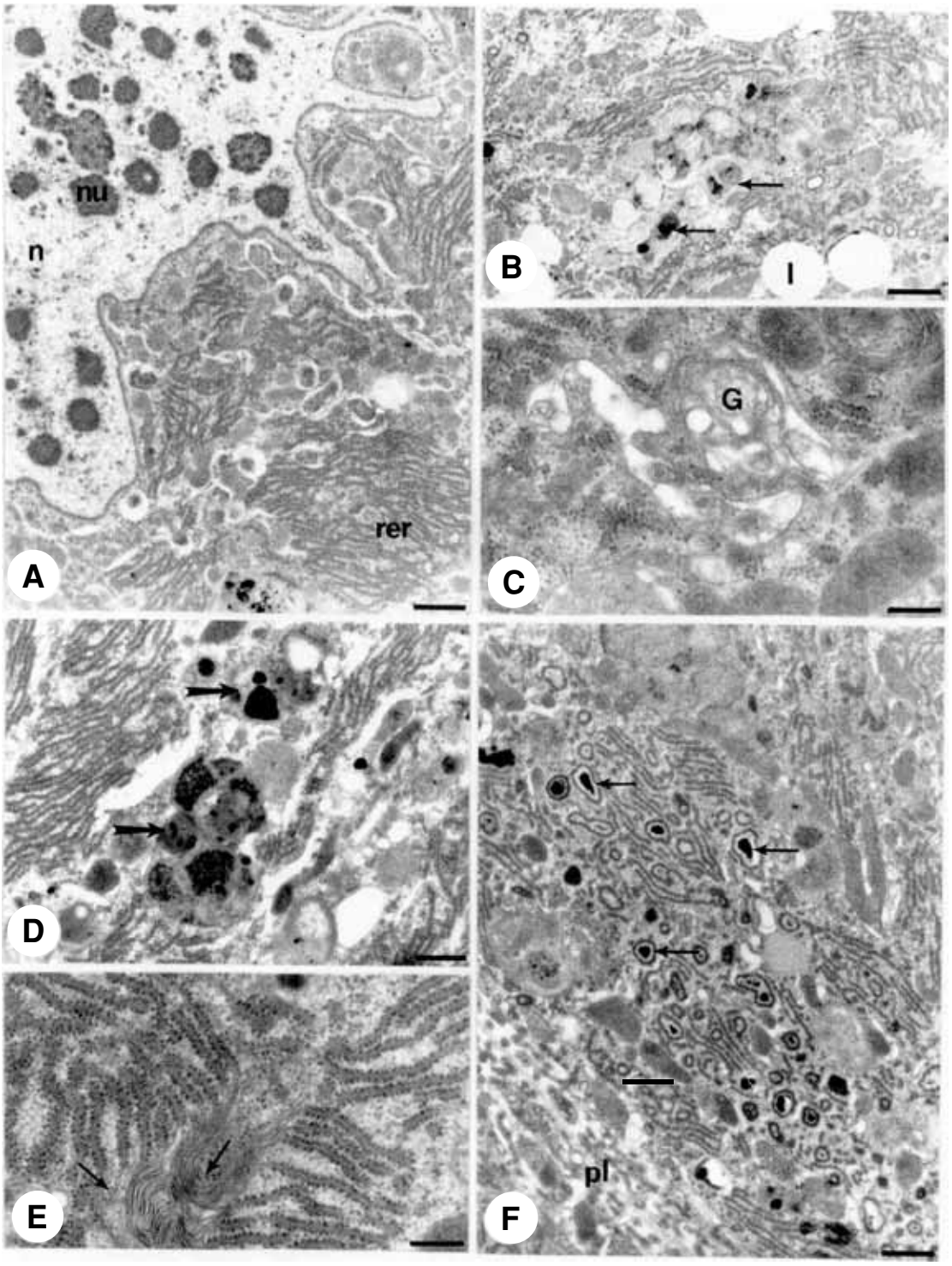

Figure 3. TEM of trophocytes in a physogastric queen of *M. q. anthidioides*. **A)** Fat body cell of the parietal compartment showing part of a large, irregular nucleus (**n**) with numerous nucleoli (**nu**). A well-developed rough endoplasmic reticulum (**rer**) is present in the cytoplasm. Bar = 1 µm. **B)** Presence of rough endoplasmic reticulum and lipid droplets (**l**), along with various types of granules (**arrows**). Bar = 1.5 µm. **C)** Golgi apparatus (**G**) in trophocytes. Bar = 0.2 µm. **D)** Autophagic structures (**arrows**) in the visceral compartment of trophocytes. Bar = 0.8 µm. **E)** Cluster of rough endoplasmic reticulum profiles typical of trophocytes (**arrows**). Bar = 0.3 µm. **F)** Typical granules found in trophocytes. Dense deposits, possibly representing the accumulation of iron (**arrows**), surrounded by cisternae of rough endoplasmic reticulum in the outer cytoplasm, close to the peripheral labyrinth (**pl**). Bar = 0.6 µm.

by the occurrence of multivesicular bodies [22]. Clearly, these cells are prepared for high rates of protein synthesis. Cruz-Landim [5] observed similar characteristics in the fat body of *A. mellifera* queens during egg laying. The increase in size results primarily from the development of the protein biosynthetic apparatus, which is consistent with the development of the vitellogenesis in the ovary of physogastric queens [11]. The trophocytes of the laying queen do not store secretion, and the lack of secretory granules in the cytoplasm suggest that vitellogenin is synthesized and quickly released from fat body cells. The appearance of the fat body nuclei agrees with the function of this tissue, i.e., their large size and the presence of many nucleoli indicates that these cells are polyploid and have high biosynthetic activity.

The results of this study do not allow us to attribute functional differences to fat body from the parietal and visceral compartments. The few differences observed fell within the range of variability for these parameters in physogastric queens. However, there are marked differences between the cells of virgin and physogastric queens. These findings indicate that these cells had a well-developed machinery for protein synthesis that involved trophocytes in providing vitellogenin to the egg yolk.

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