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Ultrastructural Study of the Follicular Epithelium in Oocytes of *Neopeonera villosa* Ants (Hymenoptera : Ponerinae)

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ABSTRACT

Insect oocytes are enveloped by the follicular epithelium which is simple and cuboidal, of which main functions are synthesis of vitellin membrane and chorion as well as synthesis and transport of hemolinph products. In ***Neoponera villosa*** ants workers aged less than 10 days do not present any formation of ovarian follicles (oocytes nurse cells and follicular cells) indicating that vitellogenesis starts at approximately 10 days of age.

Studies made on the participation of the follicular epithelium in ***Neoponera villosa*** showed that in Stage I the epithelium does not present the opening of intercellular spaces. In Stage II these spaces together with separation of the follicular epithelium from the Oocyte surface were observed. In Stage III two types of material were seen in the intercellular spaces; electrodense material in the basal region and compacted material in apical area as well as in follicular epithelium/oocytes interface, thus may suggest that the extraovarian material which reaches oocytes had some modification during passage through the intercellular spaces. It was also noted that the follicular epithelium spaces in the oocytes of queen are bigger than in those of workers.

KEY WORDS

Ants, Ovaries, Ponerinae, Follicular Epithelium.

INTRODUCTION

Insect oocytes are enveloped by the follicular epithelium. A series of functions have been attributed to follicular cells; vitellin membrane and chorion synthesis and the transport of hemolymph products into the oocytes. Protein transport is the mayor function of follicular cells, directly contributing to oocytes growth [7].

Anderson [1] confirmed that the participation of follicular cells in the transport of exogenous proteins into oocytes. He reported that in isolated oocytes follicular cells were unable to incorporate the vitellogenic proteins. This information, taken together with the discovery of the high permeability existing in the functional complexes between follicular cells and oocytes, supports the importance of the epithelium in vitellogenic processes [4].

The main purpose of the present study is to conduct any ultrastructural study of the follicular epithelium of workers and queens of ***Neoponera villosa*** ants in order to determine the morphological changes occuring in the epithelium.

MATERIALES AND METHODS

Workers of ***Neoponera villosa*** ants as well as winged females and fecundated queens, were collected in the campus of our institute and transfered to artificial nests and maintained in the laboratory in Biology Department, UNESP.

Workers aged 5, 15, 25, 30 and 40 days were dissected and ovaries were removed and processed for the observation of transmission electron

microscopy. Samples were fixed in 3% glutaraldehyde, postfixed in 1% OsO_4, dehydrated in graded alcohol and then embedded in Epon Araldite. Ultrathin sections were contrasted with Uranly Acetate and Lead Citrate and examined with a transmission electron microscopy Zeiss, EM-9-S2.

RESULTS AND DISCUSSION

The present study showed that workers of ***Neoponera villosa*** aged less than 10 days do not present any formation of ovarian follicle (oocytes, nurse cells and follicular cells), indicating that vitellogenesis in this insect starts at approximately 10 days of age. Camargo-Mathias [3] reported that follicles in the ovary of ***Neoponera villosa*** go through three stages; in Stage I the nurse chamber is larger than the oocytic chamber and the nucleus (germinal vesicle) is surrounded by the accessory nuclei, that probably arise by budding of the early accessory nuclei. In Stage II, the follicles present nurse chamber and oocytic chamber of similar size and the nuclei are still seen. In Stage III oocytes are larger than the nurse chambers, which are frequently absent. The oocytic nuclei are not observed in this stage (**Fig.1**). One of the most frequent changes observed in the follicular epithelium of insect oocytes during the phase of exogenous protein incorporation is the appearance of large intercellular spaces and the separation of this epithelium from the surface of oocytes[6]. This arrangement apparentely favors the opening of a path through which the hemolymph can have rapid accesses to the surface of the oocyte, with the consequent occurrence of vitellogenic protein uptake by the oocyte.

In ***Neoponera villosa***, the follicular epithelium of Stage I oocytes does not present the opening of intercellular spaces, indicating that what occurs at this stage is not exogenous protein uptake by oocytes but rather endogenous synthesis, as suggested by the large quantity of rough endoplasmic reticulum detected in the cortical region of these oocytes (**Figs. 2A** and **2B**).

In Stages II and III, the intercellular spaces were started to observe at considerable frequency, forming the way through which the hemolimph and protein have easy accesses to the oocyte surface. The rough endoplasmic reticulum is no longer observed in oocyte cortex (**Figs. 3A** and **3B**). These datas show that in these stages the follicular epithelium must actively participate in the uptake of extraovarian proteins. Evidence for this was the fact that the cytoplasm of follicular cells presents a well developed rough endoplasmic reticulum. This fact seems to confirm that the participation of follicular cells in the uptake of extraovarian proteins starts in Stage II oocytes.

Increased number and size of intercellular spaces were observed in the follicular epithelium of Stage III oocytes (**Figs.4A, 4B** and **4C**). In this stage, two types of materials were observed in the intercellular spaces. In the basal region there is electrodense material of flocculent appearance, and in the apical region and in the follicular epithelium/oocyte interface there is material with compact appearance and greater electrodensity. These observations lead to conclude that the extraovarian material that reaches through the intercellular spaces of follicular epithelium suffers some kinds of modifications during its passage, supporting the possibility that follicular cells synthesize some products which may probably act in the process of protein uptake, as suggested by Bast and Telfer [2], Huebner [4] and Huebner and Injeyan [5]. Other investigators have proposed that the follicular cell layer would therefore function as a barrier of regular permeability which determines the flow and the type of proteins incorporated into the oocyte. Wyatt and Pan [7] concluded that a product secreted by the follicular cells of ***Cecropia*** may interact with vitellogenin and/or stimulate vitellogenic incorporation into the oocytes by pinocytosis and the same process may be occuring in ***Neoponera villosa*** oocytes.

Fig.1. Representation of a total ovariole of ***Neoponera villosa*** ants showing the follicles at Stages I, II and III according Camargo-Mathias (1993).

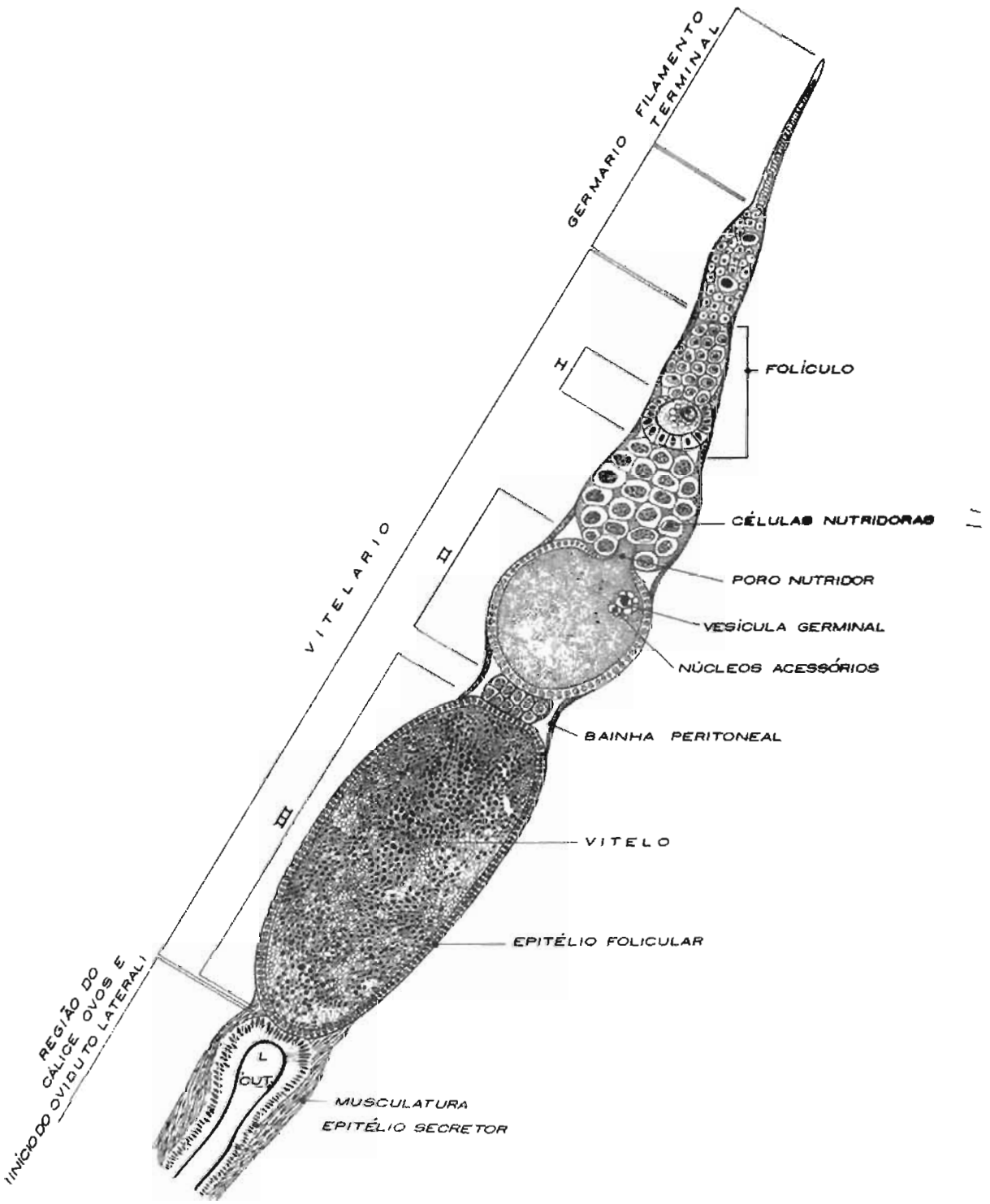
GERMÁRIO
FILAMENTO TERMINAL
FOLÍCULO
CÉLULAS NUTRIDORAS
PORO NUTRIDOR
VESÍCULA GERMINAL
NÚCLEOS ACESSÓRIOS
BAINHA PERITONEAL
VITELO
EPITÉLIO FOLICULAR
MUSCULATURA
EPITÉLIO SECRETOR
VITELÁRIO
REGIÃO DO CÁLICE OVOS E INÍCIO DO OVIDUTO LATERAL
I
II
III
IV
L
CUT

Figure 1

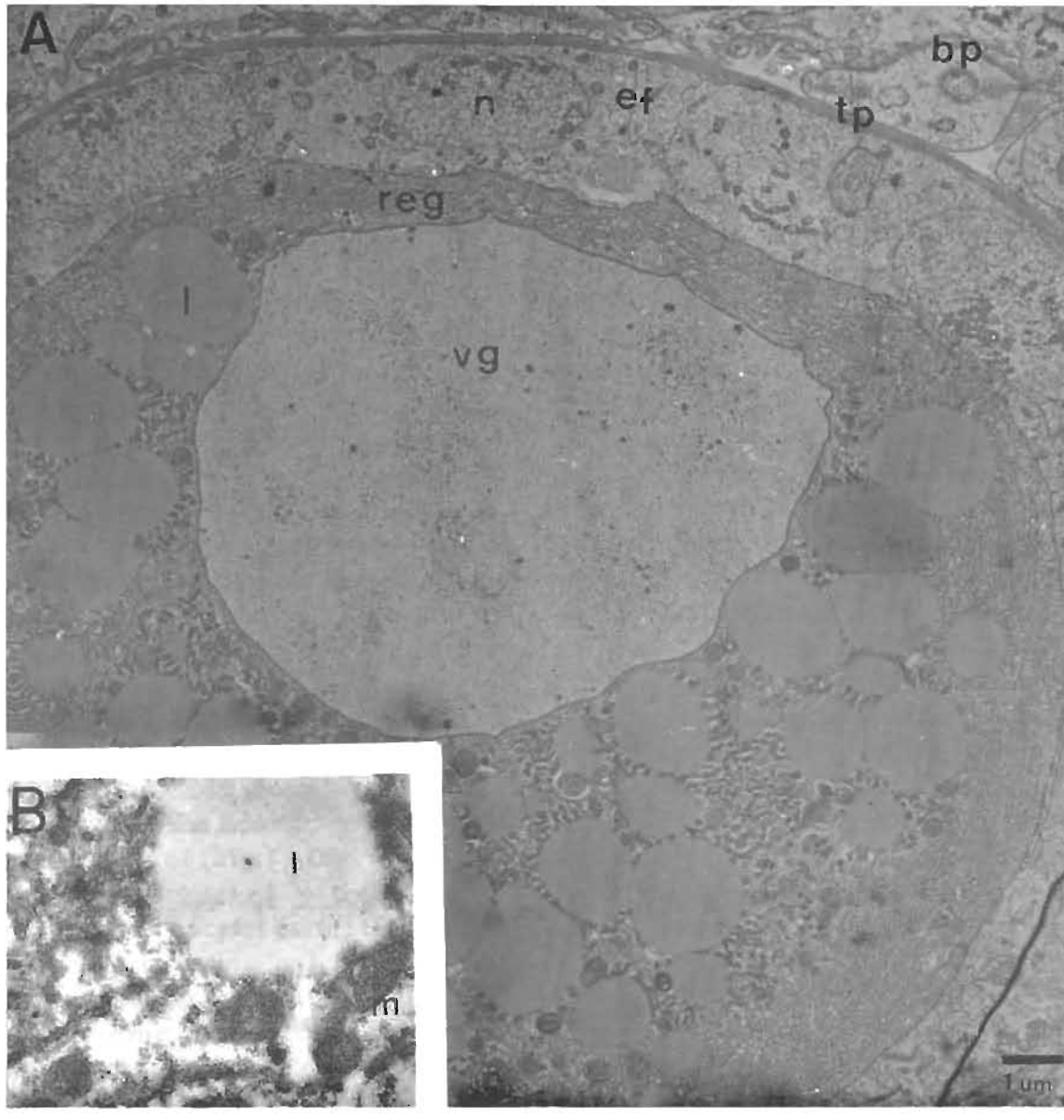

Fig.2. Ultrastructural aspects of oocytes at Stage I. **A**. General aspect. **B**. Lipid spheres (l) rounded by mitochondrias (**m**). **tp**:tunica propria. **ef**:follicular epithelium. **l**:lipid. **vg**:germinal vesicle. **n**:follicular cell nuclei. **bp**:peritoneal sheath. **Reg**: rough endoplasmic reticulum.

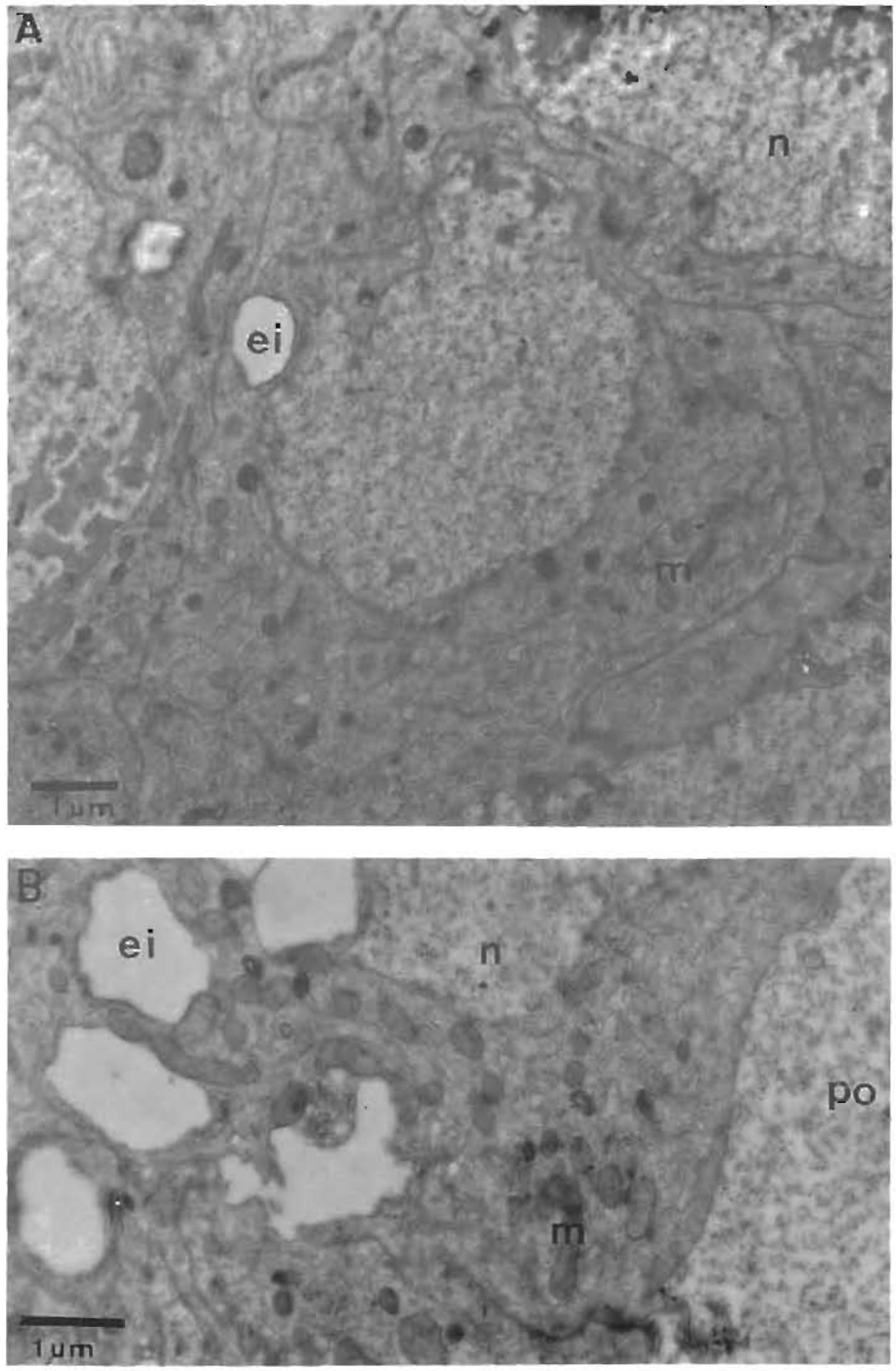

Fig.3A and **3B** Ultrastructural aspects of follicular epithelium of oocytes in Stage II showing the begining of intercellular spaces opening (**el**). **m**:mitochondria. **n**: follicular cell nuclei. **po**:oocyte cytoplasm.

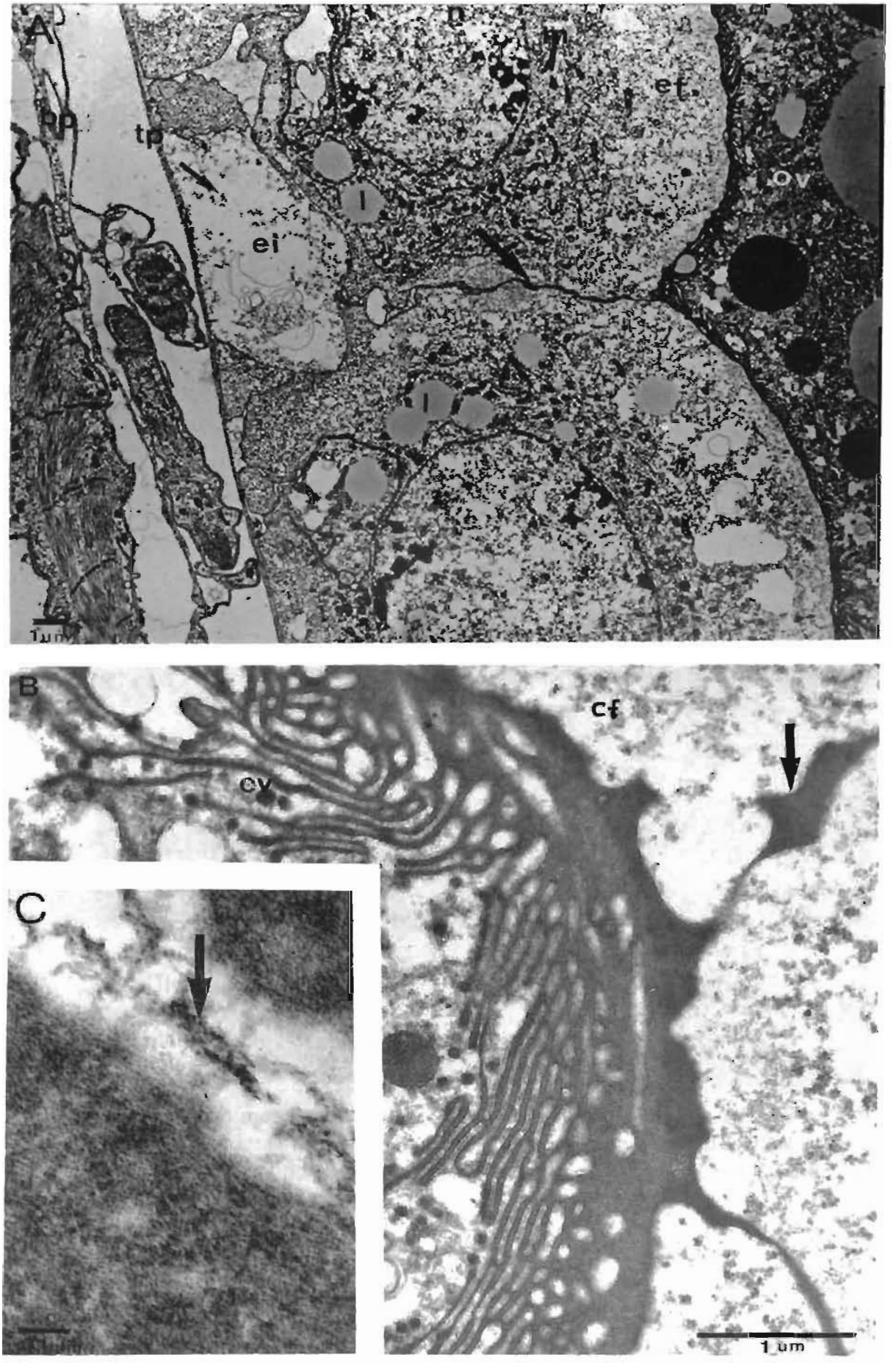

Figure 4

Fig.4. Ultrastructural aspects of oocyte in Stage III. **A.bp**: peritoneal sheath. **tp**:tunica propria. **ei**:intercellular space. **n**:follicular cell nuclei. l:lipid. **ov**:oocyte. **arrow**:electrodense material. m:mitochondria. **B.** Detail of interface of follicular epithelium/oocyte. **cf**: follicular cell arrow: electrodense material. **cv**:coated vesicles. **C.** Detail of floculent material (**arrow**) at intercellular spaces of follicular epithelium.

RESUMEN

Los oocitos de insectos estan envueltos por un epitelio folicular, el cual, es simple y cuboidal y tiene como funciones principales la síntesis de la membrana vitelina y el corión, así como la síntesis y transporte de productos hemolinfáticos (proteinas). Las hormigas obreras de ***Neoponera villosa*** con menos dc 10 días de edad, no presentan la formación de los folículos ováricos (oocitos, células nodrizas y foliculares), indicando esto que la vitelogénesis comienza a los 10 días de edad. Estudios de participación del epitelio folicular de Neoponera villosa, demuestran que en oocitos del estadio I, el epitelio no presenta la apertura de espacios intercelulares. En el estadio II, estos espacios comienzan a observarse conjuntamente con la separación del epitelio folicular de la superficie del oocito. En el estadio III, se observan dos tipos de materiales entre los espacios intercelulares: un material electron-denso en la región basal y un material compacto en la región apical, así como también, en la interfase oocito-epitelio folicular. Este último arreglo sugiere que el material extraovárico que alcanza a los oocitos sufre algún tipo de modificación durante el pasaje a través de los espacios intercelulares. Los espacios del epitelio folicular en la reina son mayores que en los oocitos de las obreras.

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