Mem. Inst. Oswaldo Cruz, Rio de Janeiro, Vol. *87* (3): 375-380, jul./sep. 1992

ULTRASTRUCTURE OF THE OVARY OF *DERMATOBIA HOMINIS* (DIPTERA: CUTEREBRIDAE) – III. GONIAL CELL DEGENERATION

V. N. D. P. SECCO; E. A. GREGORIO; L. A. TOLEDO & E. LELLO

Instituto de Biociências, UNESP, Campus de Botucatu, 18618-000 Botucatu, SP, Brasil

We studied the ultrastructural aspects of pre-pupae and pupae ovaries of Dermatobia hominis. *Physiological degeneration of gonial cells was observed: (a) after the ovarioles differentiation, in the oogonia residing in the apical region of the ovary; (b) at the beginning of vitellogenesis, in the cystoblasts close to the terminal filament. The significance of gonial cell degeneration was correlated with the physiological changes which occur in the ovary during development.*

Key words: ovary – bot-fly – Diptera – gonial cell – *Dermatobia hominis*

The earlier phases of the ovary development in insects have not been thoroughly studied. The authors working on this subject are more interested in the main processes, the differentiation of the oocyte-nurse cell complex and the vitellogenesis (King & Aggarwal, 1965; King et al., 1968; 1982; King, 1970, 1975; Mathew & Rai, 1976; Rousset, 1978; Mohanty, 1981; Bilinski, 1983). However, the knowledge of ovarian morphology prior to these events would give a better understanding of its physiology.

Ultrastructural studies of the ovary of *Dermatobia hominis* during the 3rd larval instar (Gregorio et al., 1990a) showed that the gonial cells are scattered among the interstitial somatic cells all over the ovary. Ovarioles differentiation and the origin of its tunica propria was observed in 4-days old pre-pupae (Gregorio et al., 1990b). Continuing these studies we are now presenting the physiological degeneration of the gonial cells in two moments of the ovarian development in *D. hominis*.

MATERIALS AND METHODS

Pre-pupae and pupae of *D. hominis* were dissected in insect saline solution under stereomicroscope. The ovaries were fixed in 2.5% glutaraldehyde in 0.1 M buffer phosphate pH 7.3, post fixed in 1% osmium tetroxide in the same buffer, dehydrated in acetone and embedded in Araldite. The ultrathin sections were analysed under transmission electron microscope.

RESULTS

Gonial cells degeneration was observed in two well defined periods of the ovary development:

Immediately after ovariole differentiation: from the 4-day old pre-pupae on, as the ovarioles were defined, most of the remainning oogonia at the apical region of the ovary showed different degrees of degeneration. Some of them exhibited just an increase in the cytoplasmic electron density and others besides presented nuclear alterations; these cells were surrounded by cytoplasmic processes of the neighbour interstitial somatic cells (Fig. 1A). Completely degenerated gonial cells were also observed inside cytoplasmic somatic cell vacuoles (Fig. 1B). In 7-day old pupae all the apical oogonia were in the final steps of degeneration, and they appeared as electron dense material inside huge irregular vacuoles (Fig. 2A). In 9-day old pupae these vacuoles were ocasionally found and the interstitial somatic cells had typical autophagic ones (Fig. 2B).

At the beginning of the vitellogenesis: in the ovary of 17-day old pupae, the cystoblasts close to the terminal filament, presented an increase in the cytoplasm electron density

Supported by FAPESP (Proc. 84/2979-0; CNPq (Proc. 300755/85-BM) and FIPEC (Proc. 1483/4).

Received 2 December 1991.
Accepted 22 January 1992.

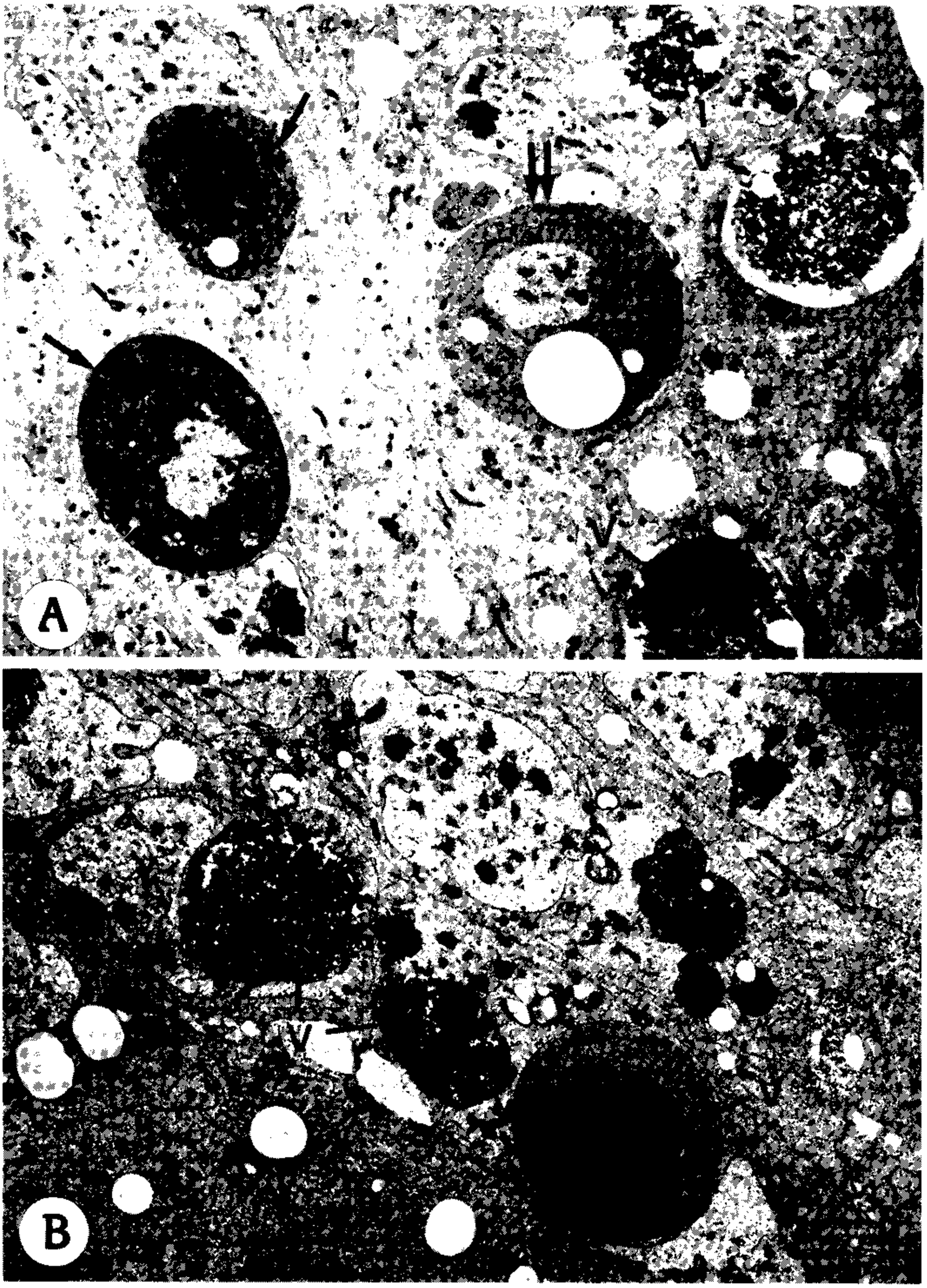

Fig. 1: A – apical region of the ovary in 4-day old pre-pupa. Oogonia surrounded by interstitial somatic cells, exhibiting either the usual morphology (double arrow) or an increase in the cytoplasm electron density (arrows); vacuoles (V) containing complete degenerated oogonia. X 5900. B – apical region of the ovary in 5-day old pre-pupa. Vacuoles (V) with oogonia debris into the interstitial somatic cells. X 6900.

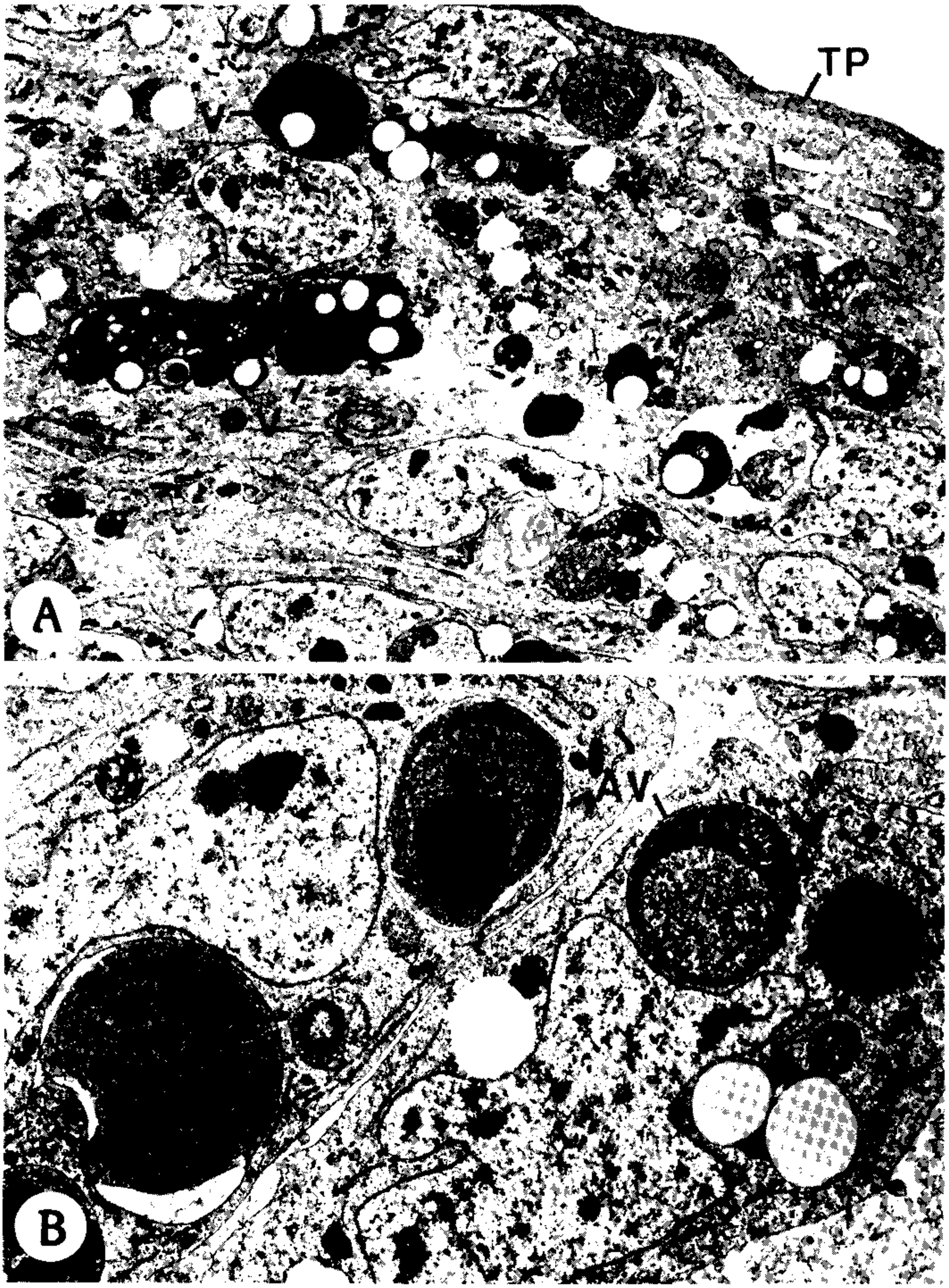

Fig. 2: A – apical region of the ovary in 7-day old pupa. Interstitial somatic cells presenting many vacuoles (V) with oogonia debris. Tunica propria (TP). X 5000. B – apical region of the ovary in 9-day old pupa. Autophagic vacuole (AV) into the interstitial somatic cell. X 15600.

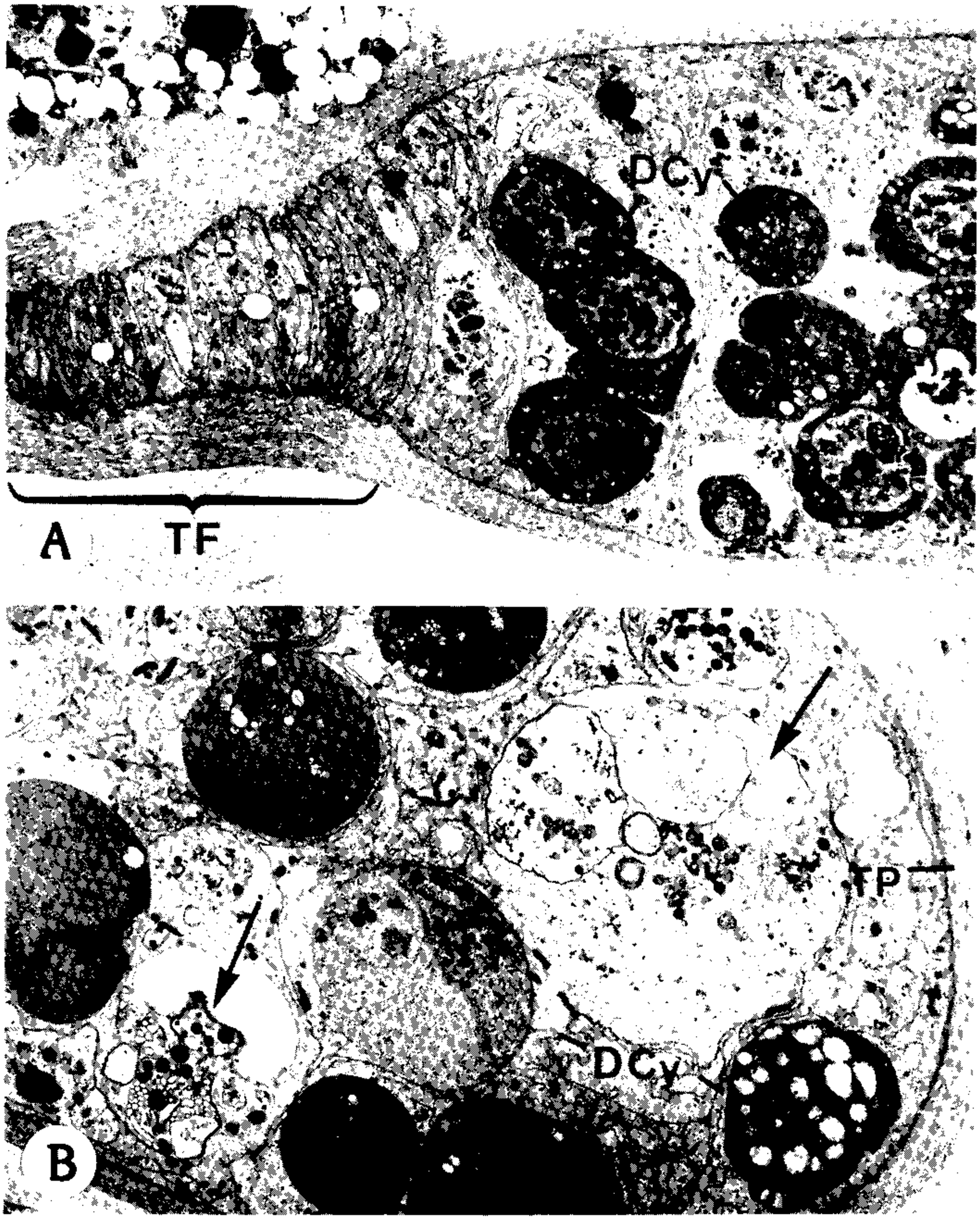

Fig. 3: A – apical region of the ovariole in 17-day old pupa, exhibiting the terminal filament (TF) and degenerated cystoblasts (DCy) at the apical region of the germarim. X 3200. B – apical region of the ovariole in 17-day old pupa. Somatic cells with huge vacuoles containing degenerated cystoblasts (DCy) and cellular debris (arrows). Tunica propria (TP). X 5900.

(Fig. 3A). Two days later these cells were detected inside the somatic cells vacuole showing different degrees of degeneration (Fig. 3B).

DISCUSSION

Gonial cell degeneration observed in the ovary of *D. hominis* is a physiological event related to the modifications of these organs during development.

In the larval period, oblong somatic cells were observed partially separating the ovary in apical and basal regions; gonial and somatic interstitial cells were scattered in both regions

(Lello et al., 1984, 1985; Gregorio et al., 1990a). The intense degenerative process observed in the apical region of the ovary starts on 4-day old pre-pupae. The oogonia are the first affected cells and this event is coincident with the formation of the ovarioles and its tunica propria. We believe that this tunica propria separates the ovary in two portions permiting two different micro enviroments. The gonial cells in these regions would receive different type and/or quantities of stimulus, thereafter showing different behaviour: cells from the apical region degenerated and the ones from the basal portion developed in cytoblasts. The final digestion of these oogonia is carried out by the interstitial somatic cells; they show huge vacuoles containing gonial cell debris in 7-day old pupae. Around the 9th day of pupation when apparentely oogonia digestion is over, the somatic cells from the apical region of the ovaries start themselves to degenerate as they present autophagic vacuoles.

Dermatobia hominis has polytrophic meroistic ovaries and the vitellogenesis starts in the basal cysts of the germarium (Lello et al., 1985). We observed in 17-day old pupae a second wave of gonial cell degeneration affecting the cystoblasts close to the terminal filament. This phenomenon is concomitant with the initiation of vitellogenesis. In principle, vitellogenesis could be iniciated in the developed oocyte by the availability of blood vitellogenin (Telfer & Kulakosky, 1984) and by the adquired competency of the oocytes to single out this protein from the haemolymph (Roth et al., 1976; Giorgi, 1979). It is also known that the synthesis and uptake of vitellogenin are triggered by juvenile hormone in the haemolymph (Sakurai, 1977; Giorgi & Macchi, 1980; Jowett & Postlethwait, 1980; Rankin & Jackle, 1980) and that the ecdysone and ecdysteroids may also play a role in these processes, as it was shown in many Diptera (Hagedorn et al., 1975; Postlethwait & Handler, 1979; Huybrechts & De Loof, 1981, 1982). There is no information on the endocrine regulation of the *D. hominis* ovary development. We believe that the hormonal control is responsible for both, the iniciation of vitellogenesis and the degeneration of the apical cystoblasts. The significance of cystoblast degeneration may be related to the life cycle of this species. The adult phase of *D. hominis* is of short duration just for reproduction; matting occurs few hours after the emergence of adult and the female starts ovoposition two days later (Banegas & Mourier, 1967). Therefore, the less developed cystoblasts that would have no time to complete their oogenesis will degenerate.

ACKNOWLEDGEMENTS

To Mrs Maria E. L. Peres and Miss Maria H. Moreno for their skillful services.

REFERENCES

BANEGAS, A. D. & MOURIER, H., 1967. Laboratory observations on the life history and habits of *Dermatobia hominis* (Diptera: Cuterebridae). I. Mating behavior. *An. Entomol. Soc. Amer., 60:* 867-881.

BILIŃSKI, S., 1983. Differentiation of the oocyte and nurse cells in an apterygote insect *(Campodea). Tissue & Cell, 15:* 965-973.

GIORGI, F., 1979. *In vitro* induced pinocytotic activity by a juvenile hormone analogue in oocytes of *Drosophila melanogaster. Cell Tiss. Res., 203:* 241-247.

GIORGI, F. & MACCHI, F., 1980. Vitellogenesis in the stick insect *Carausius morosus* I. Specific protein synthesis during ovarian development. *J. Cell Sci., 46:* 1-16.

GREGORIO, E. A.; SECCO, V. N. D. P.; TOLEDO, L. A. & LELLO, E., 1990a. Ultrastructure of the ovary of *Dermatobia hominis* (Diptera: Cuterebridae). I. Development during the 3rd larval instar. *Mem. Inst. Oswaldo Cruz, 85:* 305-313.

GREGORIO, E. A.; SECCO, V. N. D. P.; TOLEDO, L. A. & LELLO, E., 1990b. Ultrastructure of the ovary of *Dermatobia hominis* (Diptera: Cuterebridae). II. Origin of the ovariole tunica propria. *Mem. Inst. Oswaldo Cruz, 85:* 315-320.

HAGEDORN, H. H.; O'CONNOR, J. D.; FUCHS, M. S.; SAGE, B.; SCHLAEGER, D. A. & BOHM, M. K., 1975. The ovary as a source of ecdysone in an adult mosquito. *Proc. Natl Acad. Sci. USA, 72:* 3255-3259.

HUYBRECHTS, R. & DE LOOF, A., 1981. Effect of ecdysterone on vitellogenin concentration in haemolymph of male and female *Sarcophaga bullata. Int. J. Invert. Reprod., 3:* 157-168.

HUYBRECHTS, R. & DE LOOF, A., 1982. Similarities in vetellogenin and control of vitellogenin synthesis within the genera *Sarcophaga, Calliphora, Phormia* and *Lucilia* (Diptera). *Comp. Biochem. Physiol., 72:* 339-344.

JOWETT, T. & POSTLETHWAIT, J. H., 1980. The regulation of yolk polypeptide synthesis in *Drosophila* ovaries and fat body by 20-hidroxyecdysone and a juvenile hormone analogue. *Devl. Biol., 80:* 225-234.

KING, R. C., 1970. *Ovarian development in Drosophila melanogaster.* Academic Press, New York and London, p. 227.

KING, R. C., 1975. The cell cycle and cell differentiation in the *Drosophila* ovary, p. 85-109. In J. Reinert & H. Holtzer (eds). *Results and problems in cell differentiation.* Springer-Verlag, Berlin.

KING, R. C. & AGGARWAL, S. K., 1965. Oogenesis in *Hyalophora cecropia*. *Growth, 29:* 17-83.

KING, R. C.; AGGARWAL, S. K. & AGGARWAL, V., 1968. The development of the female *Drosophila melanogaster* reproductive system. *J. Morph., 124:* 143-166.

KING, R. C.; CASSIDY, J. C. & ROUSSET, A., 1982. The formation of clones of interconnected cells during gametogenesis in insects, p. 3-31. In R. C. King & H. Akai (eds). *Insect ultrastructure,* Plenum Press, New York.

LELLO, E.; GREGORIO, E. A. & TOLEDO, L. A., 1985. Desenvolvimento das gônadas de *Dermatobia hominis* (Diptera: Cuterebridae. *Mem. Inst. Oswaldo Cruz, 80:* 159-170.

LELLO, E.; TOLEDO, L. A. & GREGORIO, E. A., 1984. Diferenciação morfológica das gônadas em larvas de *Dermatobia hominis* (Diptera: Cuterebridae. *Mem. Inst. Oswaldo Cruz, 79:* 211-219.

MATHEW, G. & RAI, K. S., 1976. Ring canals in the ovary follicles of *Aedes aegypti*. *Ann. Entomol. Soc. Amer., 69:* 662-665.

MOHANTY, S. C., 1981. Growth of the vitellarium during oogenesis in the fly *Lucilia cuprina* (Diptera). I. Oocyte-nurse cell interrelation. *Growth, 45:* 90-99.

POSTLETHWAIT, J. H. & HANDLER, A. M., 1979. The roles of juvenile hormone and 20-hydroxy-ecdysone during the vitellogenesis in isolated abdomen of *Drosophila melanogaster*. *J. Insect. Physiol., 25:* 455-460.

RANKIN, M. A. & JACKLE, H., 1980. Hormonal control of vitellogenin synthesis in *Oncopeltus fasciatus*. *J. Insect Physiol., 26:* 671-684.

ROTH, T. F.; CUTTING, J. A. & ATLAS, S. B., 1976. Protein transport: a selective membrane mechanism. *J. Supramol. Struct., 4:* 527-548.

ROUSSET, A., 1978. La differentiation des cellules germinales dans l'ovariole de *Chrysopa perla* (L.) (Neuroptera: Chrysopidae). *Int. J. Insect Morphol., 7:* 59-71.

SAKURAI, H., 1977. Endocrine control of oogenesis in the housefly *Musca domestica vicina*. *J. Insect Physiol., 23:* 1295-1302.

TELFER, W. H. & KULAKOSKY, P. C., 1984. Isolated hemolymph proteins as a probe of endocytic yolk formation in *Hyalophora cecropia*. *Adv. Invert. Reprod., 3:* 81-86.