*Triatoma infestans (*Klug1834*)*'s EMBRIOLOGY [II] GONADOGENESIS

Ibáñez C. I.[1], Bozzini J.P.[1], Mariano M. I.[1,2,3].

(1) Inst. Nac. De Parasitología "Dr. M. Fatala Chaben" A.N.L.I.S., Buenos aires, Argentina.
(2) Member of the research Career Nacional Research Council, Argentina.
(3) Virrey Cevallos 385, 7º, 40.(1077) Buenos Aires, Argentina.
Corresponding autor e-mail: martaimariano@gmail.com

Received: September 2007. Approved: February 2008
Published on-line: May 2009

Abstract
The present works deals with gonadogenesis during egg embryo development in *Triatoma infestans.* Optical Zeiss Photomicrospe (OM) and Transmition. Electron Microscopy Zeiss 109 (TEM) where used simultaneously, and our observations allow to affirm that between the fourth and fifth day after egg deposition primordial gonads appears before the blastocinesis starts. From the ninth day on female and male gonads can be distinguished, their structural elements; seven ovariols in the females and the same number of testis follicles in males; are clearly established as well as the distinct regions that conform mature gonads. From these days on up to the end of embryo development, sixteen to eighteen days post oviposition and even after hatching following first and second instars, no substantial gonads morphology modification occurs, on the other hand a significant size increase takes place.
Germinal cells integration with gonads sketches, their ultra-structure and the inter-relationship between these cell types have been studied.
Key Words: Embriology, Gonadogenesis, Triatominae (Hemiptera: Reduviidae)

EMBRIOLOGIA DEL *Triatoma infenstans* (Klug1834) [II] GONADOGÉNESIS

Resumen
En el presente trabajo se estudia la gonadogénesis durante el desarrollo embrionario en *Triatoma infestans*. El estudio se realizó mediante análisis simultáneo por microscopía óptica y electrónica de transmisión. (Los microscopios empleados fueron: Fotomicroscopio Zeiss I (OM) y Microscopio Electrónico de Transmisión Zeiss 109 (TEM) Las observaciones realizadas permiten afirmar que entre el cuarto, y quinto día post-ovoposición , en el desarrollo embrionario, antes de que se produzca la blastocinesis ya se encuentran los esbozos o rudimentos gonadales. Desde el noveno día se pueden distinguir perfectamente las gónadas femeninas y masculinas, pudiéndose visualizar la presencia de sus elementos estructurales constitutivos, ovariolas y folículos testiculares respectivamente en número de siete, quedando también establecidas las diferentes regiones que los conforman. Desde este momento hasta el final del desarrollo embrionario, alrededor de los días 16 a 18 post-ovoposición y aún después de la eclosión durante los períodos ninfales primero y segundo, no se opera ningún cambio sustancial en la morfología de las gónadas, observándose en cambio un marcado crecimiento.
Se estudia la integración de las células germinales con los primordios gonadales, su ultraestructura y las relaciones celulares que se establecen dentro de ellos.
Palabras clave: Embriología, Gonadogénesis, Triatominae (Hemíptera: Reduviidae)

Introduction

Triatoma infestans hemiptera: Reduviidae with hemimetabol metamorphosis, is one of the insect species carrier of *Trypanosoma cruzi*, the protozoa causing Chagas Disease in human beings.

As all hemiptera *Triatoma infestans* is a species with separate sexes. Notwithstanding that testes and ovaries are discernable at the adult state, a relationship between some constitutive parts of male and female systems can be established (1, 2).

Triatoma infestans gonads are bilateral pair organs located at the abdomen, each one consists of seven tube-like units known as ovariols in females and testes follicles in males (3, 4, 5).

In order to understand the actual structural characteristic differences between both gonads, it is important to know the precise ontogenesis for both of them.

Background data for hemiptera embryo-genesis is present (6, 7, 8, 9), but for gonads development there is much scarce information. For *Triatoma infestans* in particular, just one work is known dealing on embryo development

where only the histology is considered (10), no mention is given for the gonads, a fact that increases the interest of this work.

The present work is the firth and only dealing with gonadogenesis for Genus Triatoma. Early primary embryo-genesis follow-up (from just ovoposited egg up to hutching.), and localizing primordial gonad sketches are orderly studied up to differentiation. Optical and TEM microscopy were used simultaneously in order to recognize the first appearance of germinal cells, their participation in gonad body morphogenesis and the inter-relationship with other cell types as embryo develops.

Material and Methods

Triatoma infestans eggs used in the present study came from insects reared at an insectarium that was maintained at 27º Celsius (27 ± 1 º C). At the mentioned temperature the eggs development time from oviposition up to hatching endures from 16 to 18 days.

Early samples processed were fixed 10 hours after oviposition, further step wisely samples were processed up to 17 days after oviposition.

Feints on the egg surface corion were performed at different positions (at both egg poles, and at the center less curved surface) in order to allow the rapid penetration of Karnovsky liquid fixer that was prepared few minutes before eggs immersion. They where maintained in the fixer during the next twelve hours at a temperature below 10ºC. Later the samples were washed twice during 15 minutes each one with 0,1M Sodium Cacodilate buffer, to be post fixed with 1% Osmium tetra-oxide cacodilate buffered solution for the next 24 hours at the same temperature.

Fixed samples were dehydrated with a graded up series of ethylic alcohol, followed by two washes in propylene oxide – one hour each wash. The samples were them imbedded in poly-bed araldite resin mixture after Mollenhauer (11). For light microscopy sections 1µm to 2µm thick were obtained with a L.K.B. microtome and stained with methylene blue, azure II. They were mounted on 1,5mm thick slides with poly-bed araldite resin, to be analyzed with a Zeiss-Photomicroscope either under light field or phase contrast. Full eggs were fixed as described before, and histology sections either across a longitudinal or a transversal plane, taking into account the central long axis of the egg body, were cut.

Ultra-thin sections 60 nm thick where obtained with the same microtome, contrasted with lead citrate and uranyl acetate after Reynolds (12) and observed and micro graphed with a Zeiss 109 T. E. M. For all photographs Kodak T.Max 100 film was used as negative material; it was processed with $D19_b$ developer.

Results

As is shown in Fig. 1a the just oviposited egg has a thick corion covering its whole body, which is follow inside in close adhesion by a fecundation membrane **fc,** both are jointly separated from the plasma membrane by the so-called “peri-vitelline space”. The zone opposed to the operculum is occupied by a “bubble like” structure which determines a discontinuity in the corion, where this bubble seems to open, disconnection that also affect the fecundation membrane and the plasmalema. This membrane forms two folds that seams terminal, see Fig.1b.

Located into this egg acute end within the yolk the germinal vesicle is present as sphere body, inside it, dense granules are found either spaced out or joined in a chain like structure, they might be nuclear material ¿DNA?, although no chemical proof was performed. The yolk mass at this early development is populated by cells in active division Fig. 2. which metaphase nuclei are conspicuous.

Among the metaphase cells other in fewer number are characterized by a significative number of cytoplasmic projections; they are the so-called “vitelophages” cells that are scattered within the yolk Fig. 3a. The already

described characteristics are less evident at the operculum egg pole. Fig. 3b.

There is a cell migration toward the periplasm, it determines the made up of a disordered cell massive, particularly at the egg operculum pole. As time goes on the mentioned cells distribution turns to a better ordered giving rise to the formation of a germinal band Fig. 4.

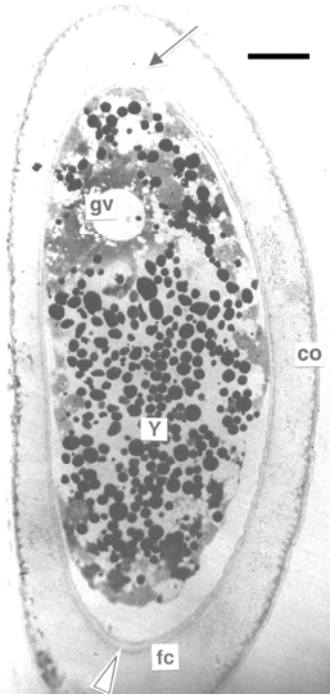

Fig. 1a. Egg one day after oviposition, showing a thick corion: ***co***, the fecundation membrane: ***fc*** is in close junction to it. Between ***fc*** and the plasma membrane the peri-viteline space is present (arrow head). At the pole opposite to the operculum a bubble like structure is present (arrow), yolk: ***y***, germinal vesicle: ***gv***.
The bar indicates 200µm

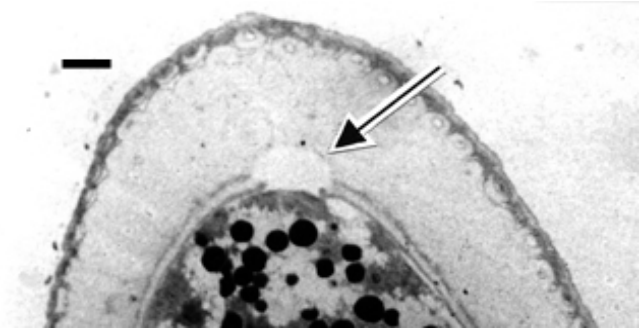

Fig 1b. Detail of Fig 1a pointing out the bubble like structure. The bar indicates 100µm

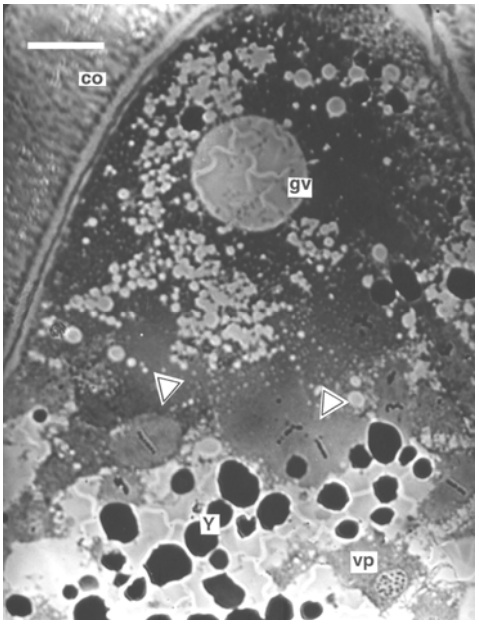

Fig. 2. One day after oviposition egg as in fig. 1, opposite to the operculum extremity. The micrograph shows the germinal vesicle: *gv*, inside it little dense granules set out a chain. Metaphase cell divisions (arrow head), yolk: *y*, corion: *co*, vitellus-phage: *vp*.
The bar indicates 80µm

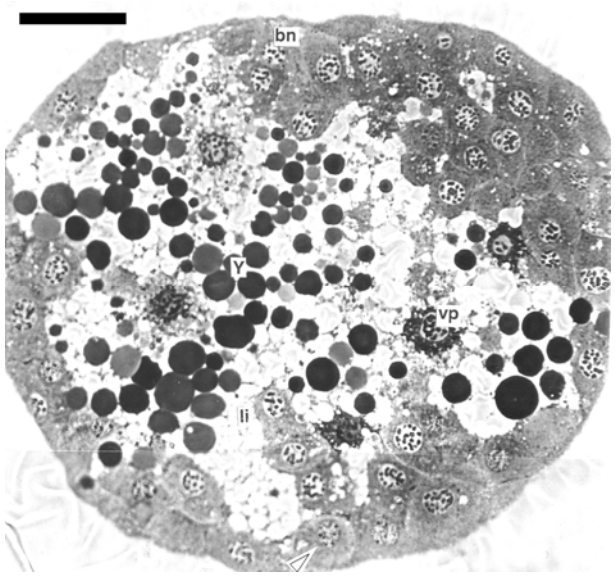

Fig. 3a. One an a half days after oviposition egg. Blastoderm nuclei: ***bn*** placed around the yolk are present. Vitelus-phage: ***vp***, lipids: ***li***, yolk: ***y***. The arrow head points primordial germinal cells.(photomontage).
The bar indicates 100 µm

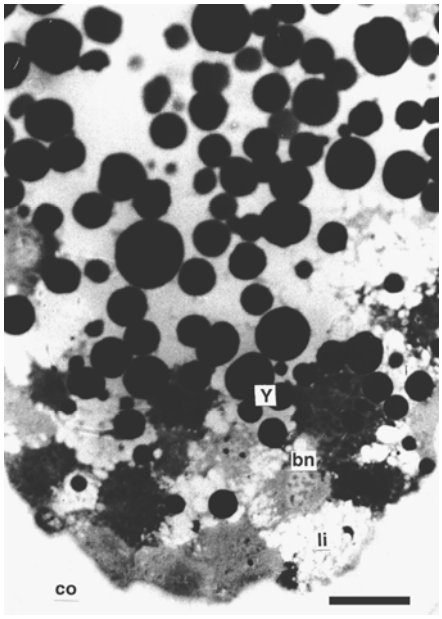

Fig. 3b. Histological section of an embryo one an a half days after oviposition; the corion: ***co*** is still present. The operculum end is shown.-Blastoderm nucleus: ***bn***, lipids: ***li***, yolk: ***y***. The bar indicates 80µm

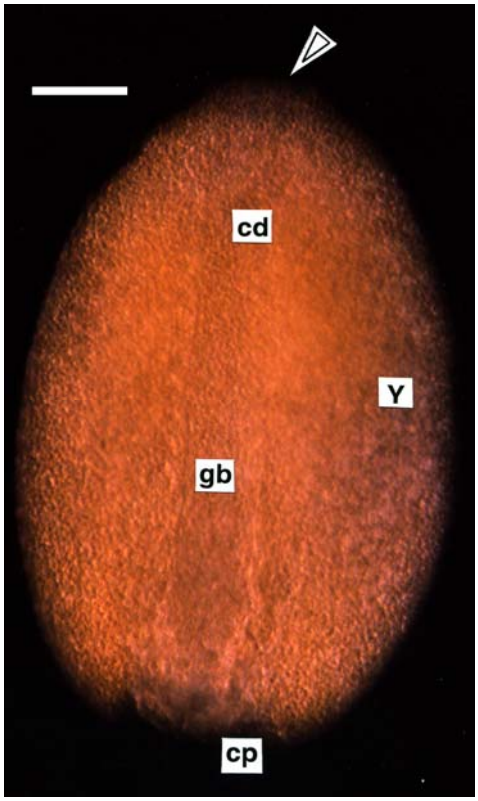

Fig. 4.- Embryo in a corion less egg two days after oviposition. The germinal band: gb is shown. The caudal zone: cd and the cephalic one: cp can be distinguished. Yolk: y, the arrow head points the operculum zone. The bar indicates 300µm

The germinal band is build up within 60 to 70 hours after oviposition. Around the third day, blasto-kinesis starts. Blasto – kinesis movements, a 180º rotation, conducts a position change of the embryo within the egg, the head or cephalic embryo end that is formed at the egg end opposite to the operculum, Fig. 5 is slowly rotated to its final position. The head points toward the operculum at the blastokinesis end Fig. 6. This wide state movement that can be observed trough the translucent corion by the ocella position, was taken by us as reference for time and space location of sketched gonads during embryogenesis.

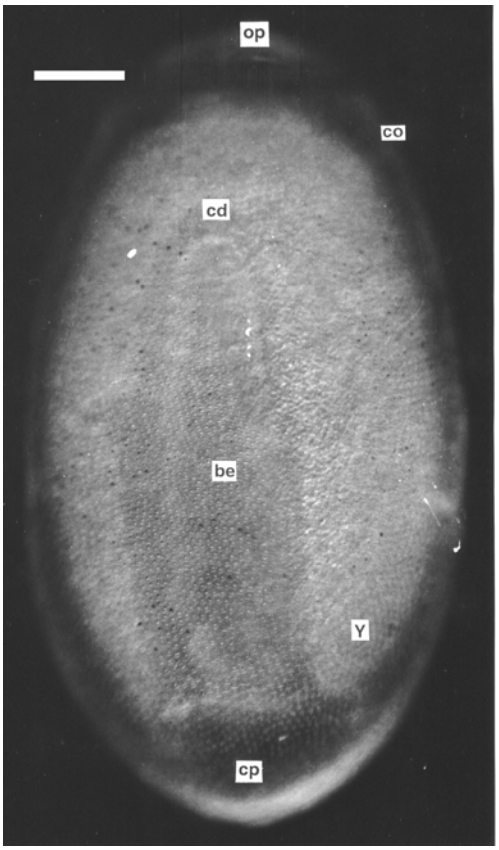

Fig. 5. Fourth day after oviposition embryo within an egg with its corion: *co*. Embryo cephalic end: *cp* points to the operculum opposite extremity *op*, while towards this egg tip the caudal end: *cd*, points. Embryo body: *be*, yolk: *y*. Compare with Fig. 6.1. The bar indicates 200µm

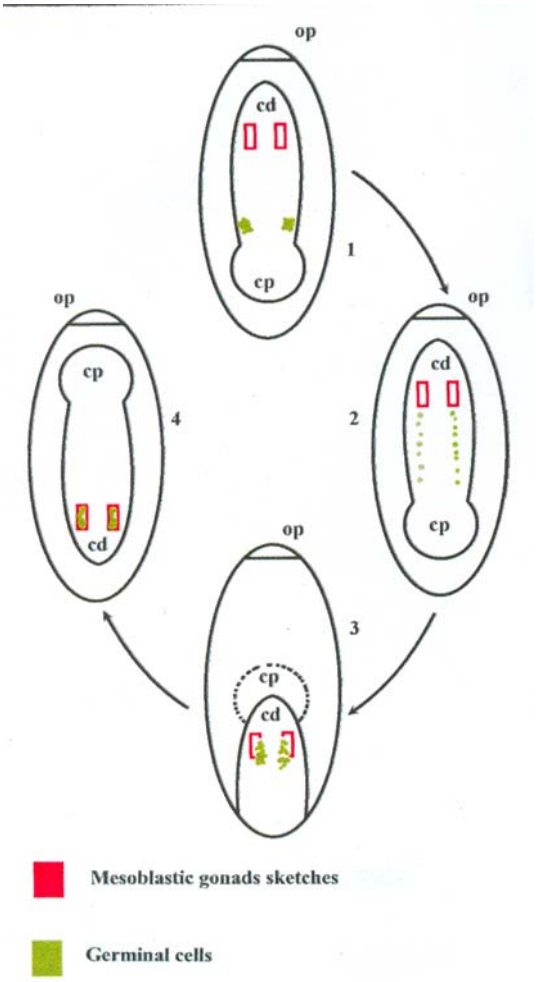

Fig. 6. Diagramed draw presenting the gonads organ-genesis as takes place while blastokinesis movements come about. 1. Between 3rd and 4th day after oviposition mesoblast gonads sketches and cell dehiscence can be observed taking form, from cephalic region blastoderm mass. 2. Between 4th and 5th day after oviposition, the germinal cells migrate toward mesoblast gonad sketches. 3. Between 5th and 6th days after oviposition, the germinal cells start their entrance into the gonad sketches. 4. 7th day after oviposition. The integration and organization of somatic and germinal cells in the gonad sketches is clearly observed. Caudal embryo tip: cd, cephalic embryo tip: cp, operculum: op

Between third and fourth days we have observed that from cell masses that are localized in cephalic or fore embryo position, cells dissociation begins. From this embryo location a displacement takes place, either in groups or isolated, also forming chains, they relocate near by the mesoderm. Figs. 7 and 8; they are the germinal cells.

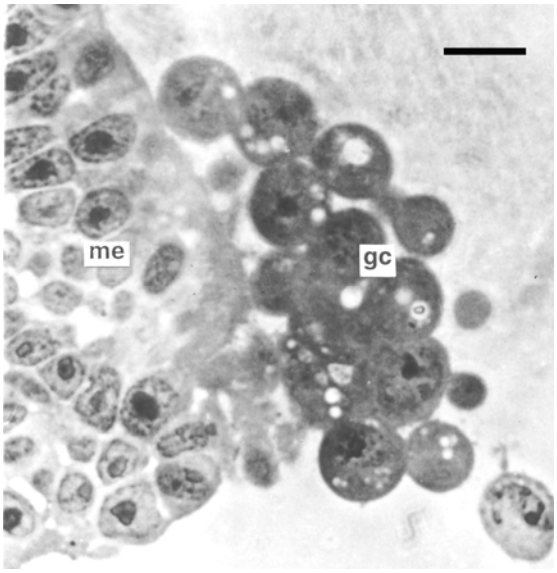

Fig.- 7.- Five days after oviposition embryo, histological section showing the stick together, agglutinated germinal cells: *gc*, during the migration. Mesoderm: *me*. The bar indicates 25 µm

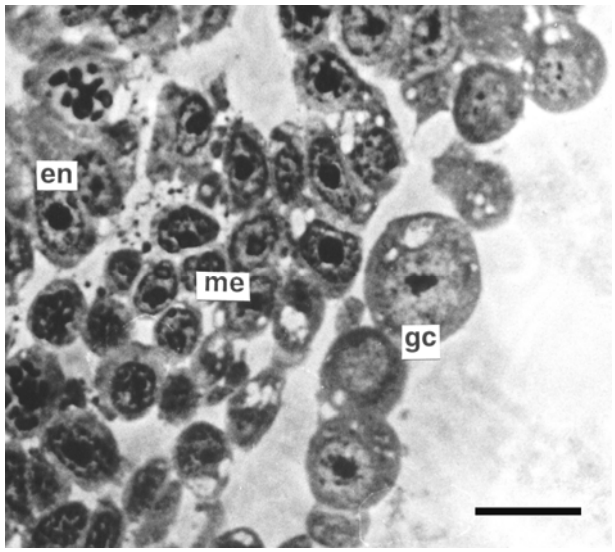

Fig. 8. Another embryo of the same type (five days after oviposition) where the migrating germinal cells: *gc*, are easily seen. Endoderm: *en*, mesoderm: *me*. Compare with Fig. 6.2. The bar indicates 25 µm

We can recognize them by their spherical body, scarce cytoplasm, large nuclei, conspicuous nucleoli, and in their cytoplasm vesicle like inclusions are found, they appear either under few large size or many small size vesicles.

The germinal cells present at the caudal end of embryo, occupies the last abdominal segments. As can be appreciated following a length frontal section of the dorsal zone Fig. 9, the germinal cells are present in symmetrical position at right and left embryo borders, they distribute giving rise to a row or lace.

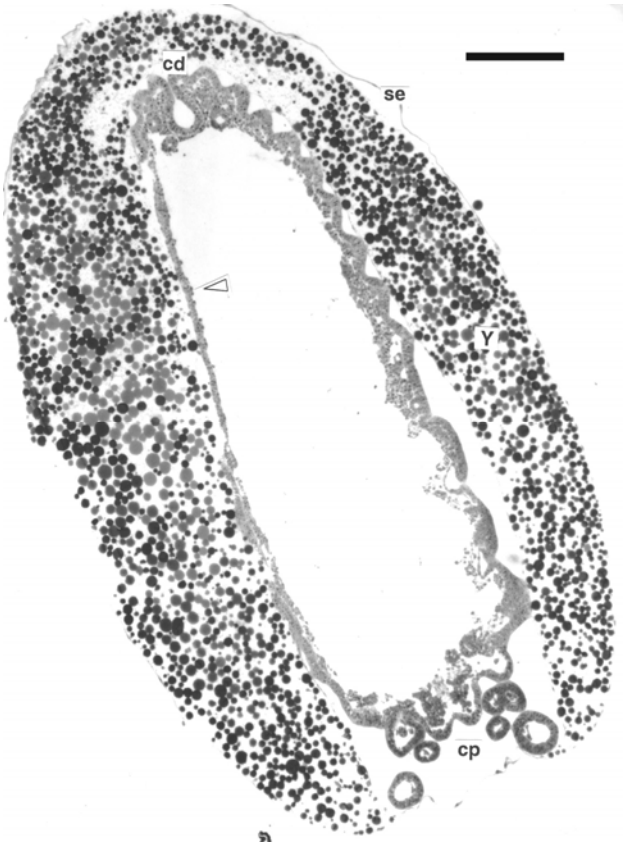

Fig. 9. Fifth day post-oviposition embryo histological section. Phase contrast micrograph. This is a frontal section although section plane is not bilateral equidistant. Cephalic region: ***cp***, caudal region: ***cd***, serose tissue: ***se***, yolk: ***Y.*** The arrow head points migrating germinal cells. Compare with Fig. 6.2. The bar indicates 205µm.

Five days after oviposition as is illustrated in Fig. 10 the embryo with a clear bilateral symmetry shows the sketches of the appendices, the early mouth apparatus, somite composed segmented body across the antero-posterior egg axis has still their cephalic end in direction opposite to egg operculum. Between fifth and sixth days, when the embryo movement and rotation is easily observed Figs. 11a and 11b, in a length section of a corion less egg, near to the developing fat-body a protruding cell cumulus is found which is the early gonads sketch or primordium, Fig. 11c. Germinal cells are now associated with the smaller somatic ones. These cells start to intermingle with the larger germinal, Fig. 11d.

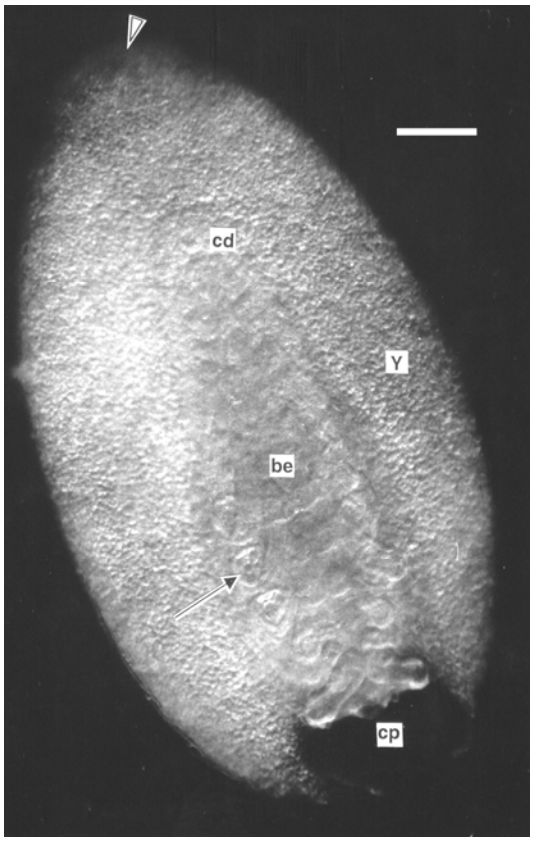

Fig. 10. Embryo between the forth and fifth after oviposition within an egg which corion was excised. The embryo body: ***be*** is observed. Cephalic region: ***cp***, caudal region: ***cd***, yolk: ***y***. The arrow points a thorax appendices rudiment. Arrow head points the operculum zone. The bar indicates 220 µm.

The sixth day presents a clear advance in embryo displacement Fig. 12. A longitudinal-frontal section of a corion deprived egg shows the caudal embryo end getting distant from the operculum. By the same period analyzing (1µm to 2µm) trans- or para-sagital histo-logical sections in the gonad sketch seven nearly circular structures can be identified.

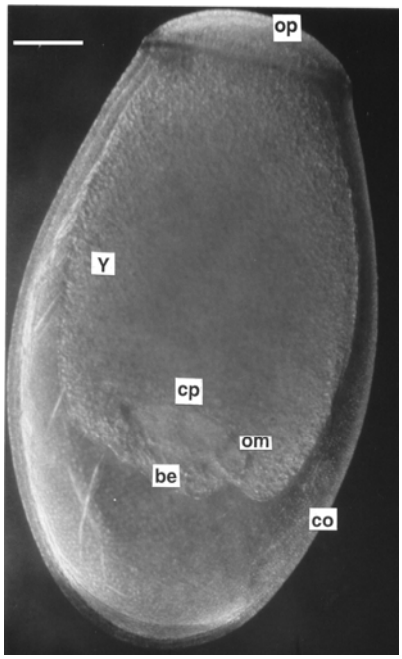

Fig 11a. Fifth day after oviposition embryo within an egg with its corion: *co*, while the blastokinesis movements have started. Embryo displacement can be follow by the position of the cephalic region: ***cp***, with the omatidia: ***om***, turning apart from the opposite to the operculum egg tip in its rotation toward the operculum: ***op***. Yolk: ***y***, embryo body: ***be***. Compare with Fig. 6.3. The bar indicates 280µm

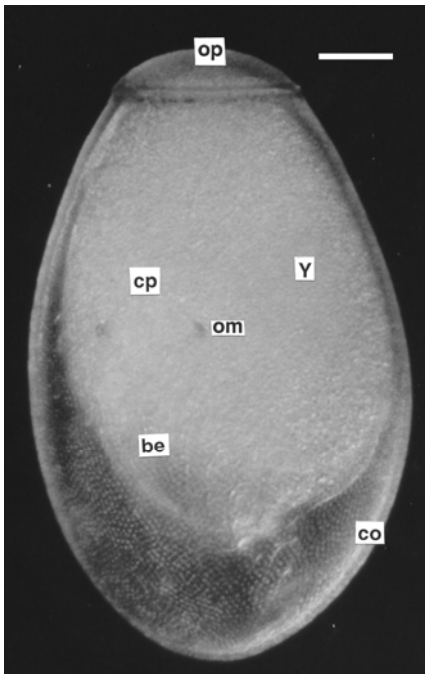

Fig. 11b. Embryo at the end of the fifth day after oviposition. The cephalic region: ***cp***, advances toward the operculum: ***op***, (It can be followed by omatidia position). Embryo body: ***be***, yolk: ***y***, corion: ***co***, omatidia: ***om***. Compare with Fig. 6_3.The bar indicates 300µm

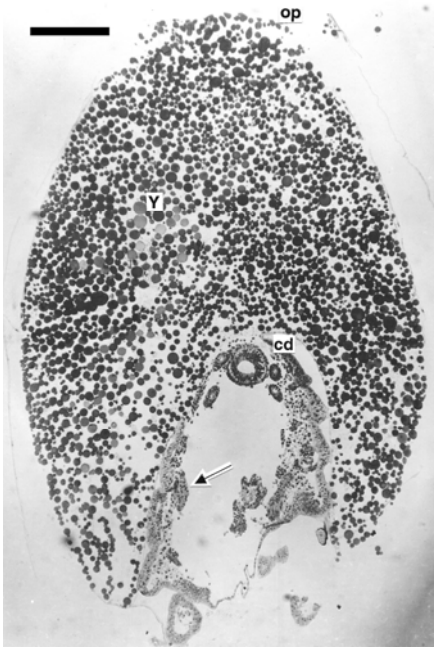

Fig. 11c. Embryo histological section sixth day post-oviposition. Phase contrast micrograph. According to the section plane orientation there is a conspicuous presence of the yolk mass, which is placed at embryo dorsal side. The embryo body occupies a reduced sector of the section, within which gonads can be localized (arrow). Caudal region: ***cd***, yolk: ***y***, operculum zone: ***op***. Compare with Fig. 6.3. The bar indicates 220µm

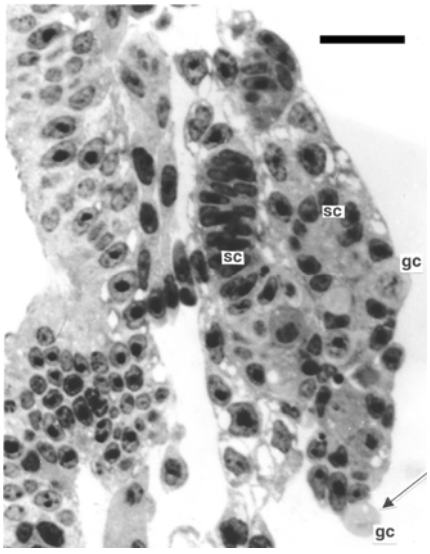

Fig. 11d. Higher magnification detail of fig. 11c. Developing gonads are clearly seen where germinal cells: ***gc*** and somatic cells: ***sc,*** can be distinguished. At the right bottom corner the arrow points a germinal cell getting into the gonad sketch. The bar indicates 20µm.

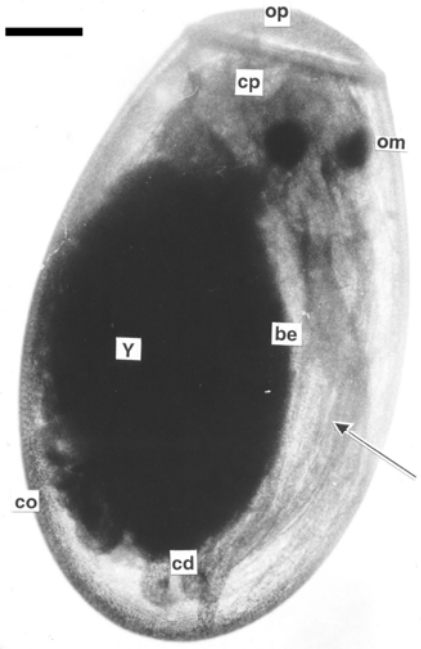

Fig. 12. Lateral view of an embryo (reaching the operculum zone) within an egg with its corion ***co***, seventh day after oviposition. Cephalic region: ***cp,*** operculum: ***op,*** omatidia: ***om,*** yolk:***Y,*** embryo body: ***be,*** caudal region: ***cd.*** The bar indicates 300µm

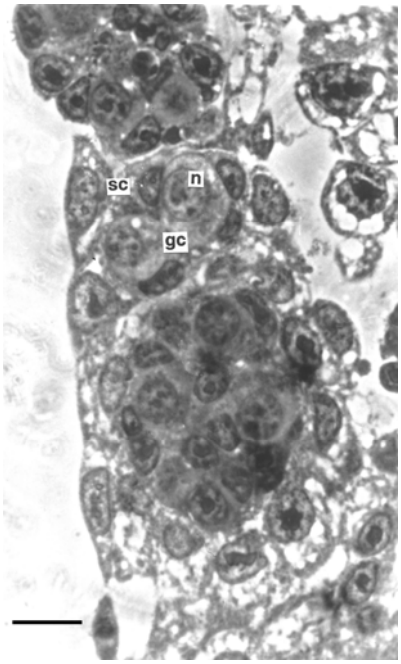

Fig. 13. Histological section of a developing gonad within an embryo, sixth days after oviposition. Germinal cells: ***gc,*** with their large size nuclei: ***n,*** and somatic cells: ***sc*** are distinguished. The bar indicates 16µm

In Fig. 13 each one of these structures contains germinal and somatic cells. The last ones can be divided into two types: some of dense cytoplasm and circular border, the second one has a less dense cytoplasm but their section rim seems an oblong deformed circumference. All the cells are immersed in a slack lax stroma and the whole is limited by flat cells tunica. Seven days after oviposition the blasto-kinesis is completed. The embryo has now a position 180° opposed to the former one, the cephalic end is now near to the egg operculum, and the caudal extremity directs toward the opposite end of the egg. As shown in micrograph of an egg with corion by the omatidia at the operculum pole Fig. 14. The gonads remain at a caudal dorsal position within embryo, according to the section plane direction they appear either in closness or less near to the yolk. Nevertheless they still are at close of abdomen vitellus remains as well as to the developing fat body Figs. 15a and 15b.

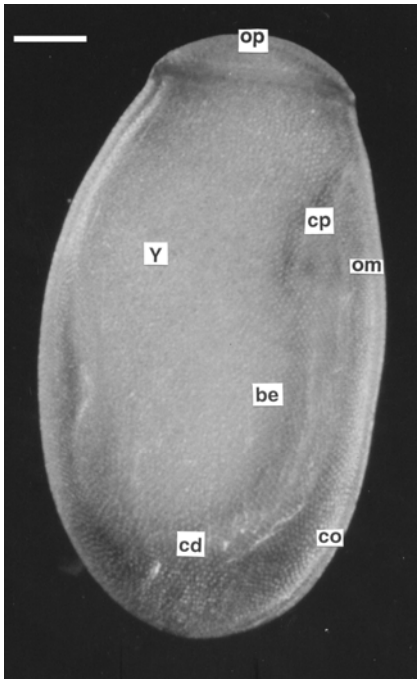

Fig. 14. Seventh day post-oviposition embryo within an egg which corion was made transparent by treatment. Blastokinesis has been completed. The embryo head, cephalic region: ***cp,*** is in coincidence with the operculum: ***op,*** while de caudal zone: ***cd,*** occupies now the opposite

egg tip. Thorax appendices can be seen (arrow). Embryo body: ***be,*** omatidia: ***om,*** corion: ***co*** yolk: ***y***. Compare with Fig. 6.4. The bar indicates 280µm

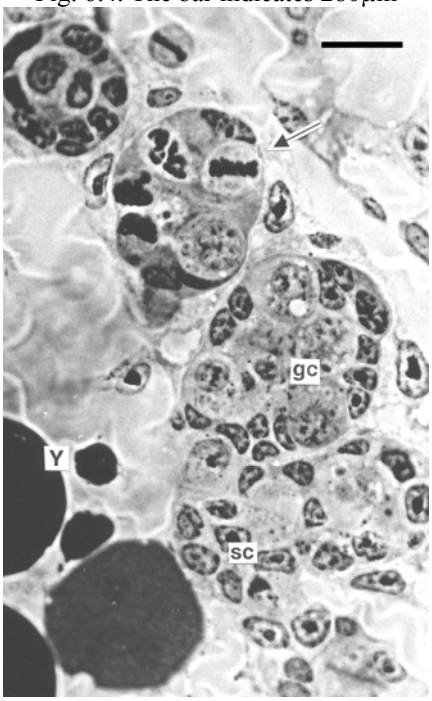

Fig.15a. Histology section of an embryo, eighth day post-oviposition. A gonad was trans-sectioned, it shows four structural units. The germinal cells: ***gc***, are positioned at the center of each structure, the somatic ones: ***sc***, are positioned around ***gc***, at each unit periphery. yolk: ***Y*** . The arrow points a mitotic cell. The bar indicates 15µm.

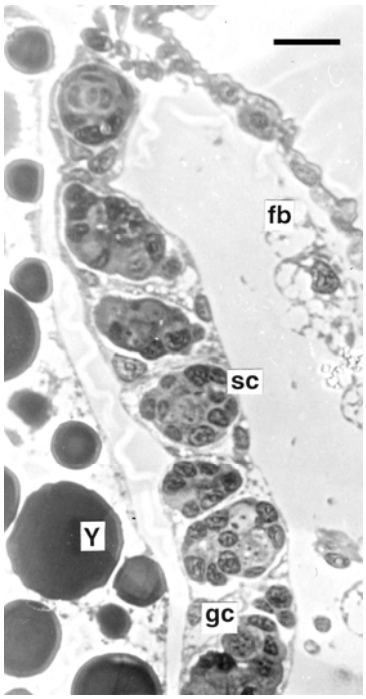

Fig. 15b. Photomicrography of a gonad in an embryo at the eighth day post-oviposition. Germinal cells: ***gc***, somatic cell: ***sc***, serous membrane: ***se***, yolk ***Y***, fat body: ***fb.*** The bar indicates 15µm.

Gonads structure present the circular shape follicle like pattern, with the cell types already described for the sixth day Fig.16.

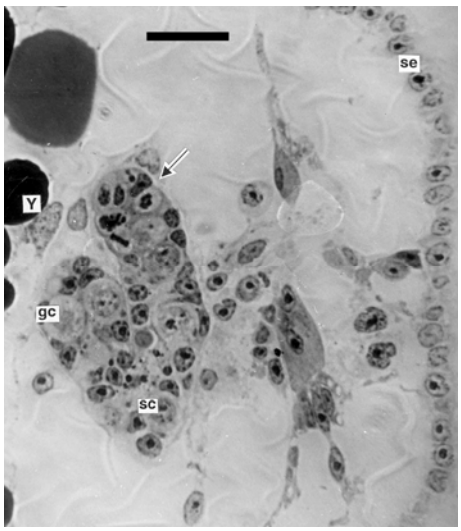

Fig. 16. Histology section from an eighth day post-oviposition embryo. A gonad has been sectioned where the complete seven structural units that compose it are distinguished. Within it the germinal cells: ***gc,*** and the somatic cells: ***sc,*** are conspicuous. Yolk: ***y.*** The bar indicates 10µm

Eight days after oviposition either frontal-longitudinal sections of caudal dorsal embryo region as well as longitudinal sagittal sections shows the presence of the digestive tube, the gonads are now in a position between this structure and the body wall Fig. 17.

The seven unitary follicle structures are now easily observed. Inside each one the new distribution started the day before are manifest, the germinal cell are now centered surrounded by the somatic cells Fig. 18. Some embryos star to show gonad primordial differences that allow us to interpret as characteristic of the female, although those differences are not yet clear cut established. As a matter of fact trans-sections of the follicles composing the gonad, cell organization seems to determine two regions, the first with a large number of

germinal cells, and a second where somatic cells organize themselves following an epithelial order. Fig. 19.

Germinal cells are now rather conspicuous, their nucleus – cytoplasm relationship is going to balance since cytoplasm volume has increased in size as compared with the nucleus. Somatic cells start to place around the germinal cells, and take a polarized position towards the extreme of primordial gonads.

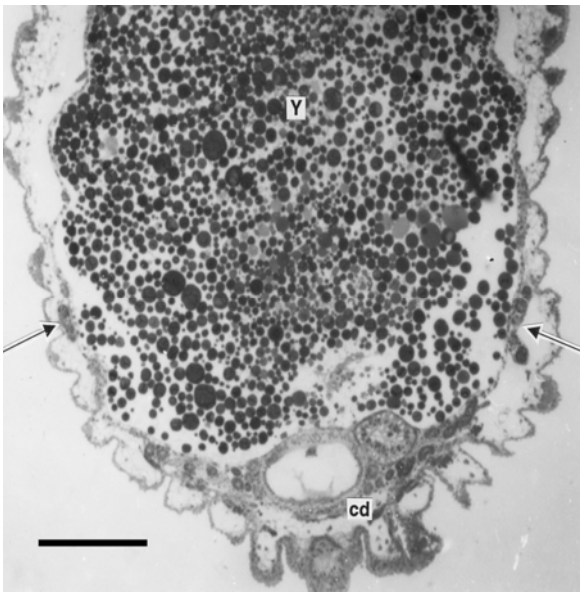

Fig. 17. Frontal longitudinal histology section from an embryo caudal region: ***cd,*** eight days post-oviposition. The gonads, pointed by the (two) arrows, are placed at the abdominal segments, yolk: ***y.*** The bar indicates 120µm.

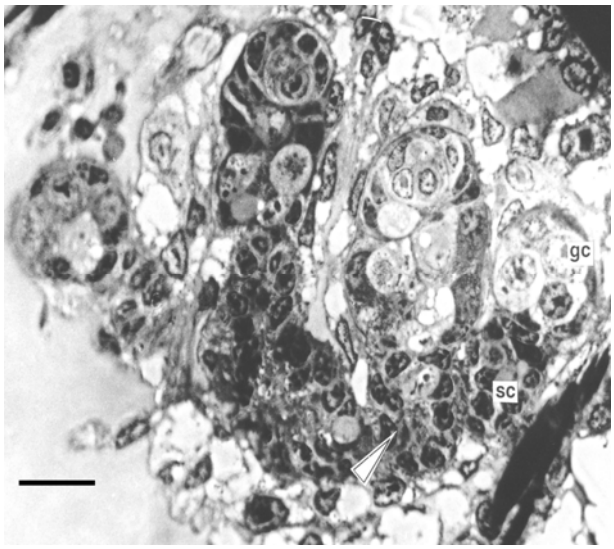

Fig. 18. Histology section of a gonad at the eighth day after oviposition. The somatic cells: ***sc*** start to develop a duct like structure, Germinal cells: ***gc.*** The bar indicates 6,5µm.

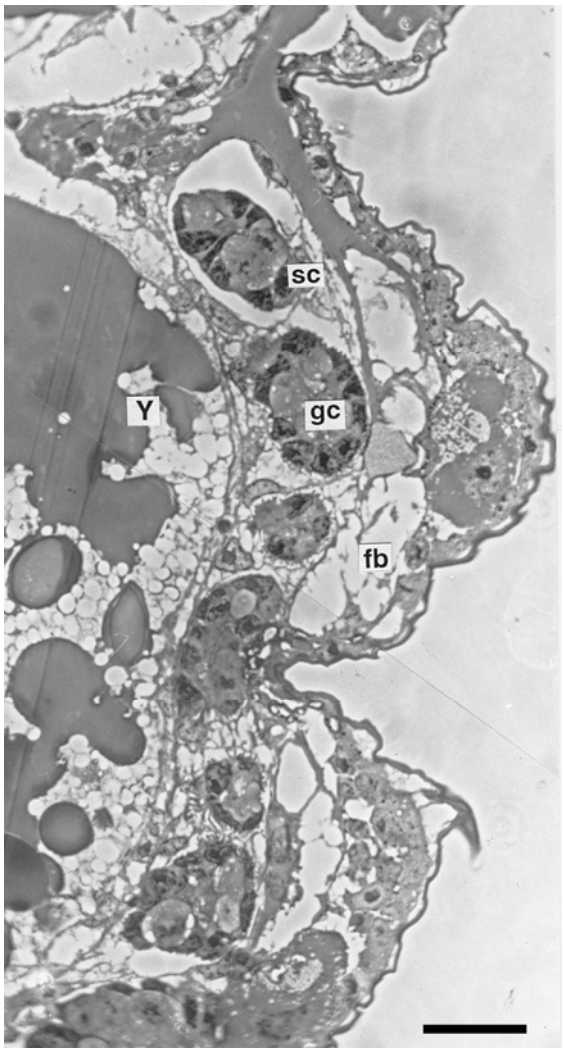

Fig. 19. Section across a male gonad from a ninth days after oviposition embryo. The seven follicles structure units that compose the testis body are easily perceived. In any one of them are distinguished the germinal cells: ***gc,*** and the somatic cells: ***sc.*** Fat body: ***fb,*** yolk: ***Y.*** The bar indicates 15 µm.

The ultra-structure of germinal cells show large nuclei, endoplasmic reticulum (ER) not yet fully developed and a well expanded micro-tubes system is present at the cytoplasm. Germinal and somatic cells get in touch with themselves crosswise cytoplasmic bridges. Fig. 20a and 20b.

At the ninth day of embryo development differences between female and male gonads are precise. Both at the ovariole as well as at testis follicles the distinct regions that distinguish them are fully established. They consist of the fundamental body where the germinal and somatic cells are located and the specialized tissue that will conduct the mature gametes in the adult state.

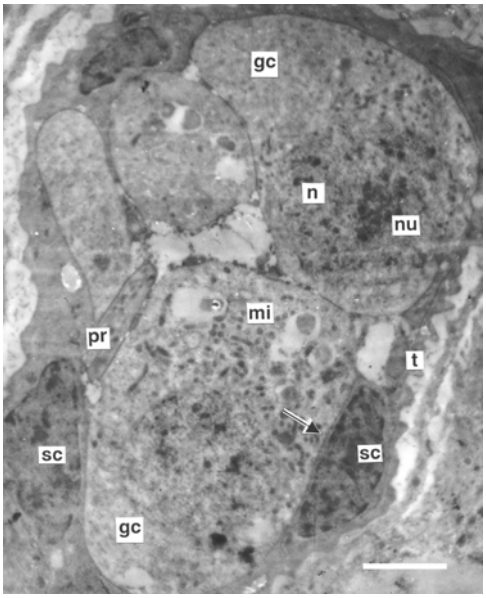

Fig. 20a. TEM thin section from gonad a ninth day post-oviposition embryo. Germinal cells: ***gc***, are surrounded by somatic cells: ***sc***. Between both cell types septata unions are present (arrow). Germinal cells show a considerable mitochondria: ***mi***, number which has a polarized position. Nucleus: ***n***, nucleolus: ***nu***, serose tunic: ***t***, cytoplasm process: ***p***r. The bar indicates 1,3 µm.

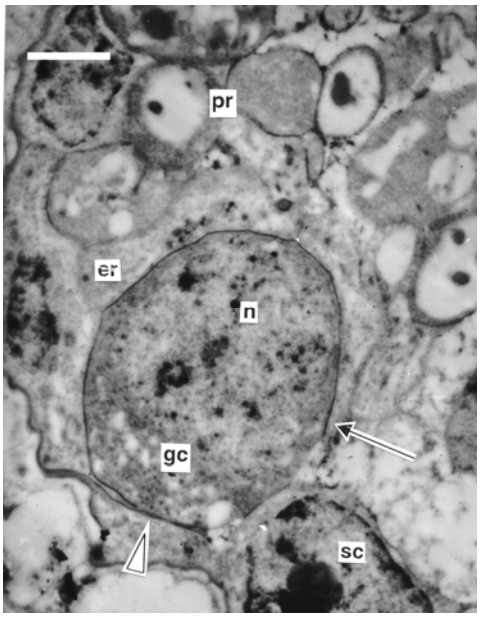

Fig. 20b. Gonad primordia TEM micrograph from a seventh day after oviposition embryo. A germinal cell: ***gc,*** with a large nucleus: ***n,*** is in close contact with somatic cells: ***sc,*** and a considerable number of cytoplasm processes: ***pr,*** adherens junctions (arrow head) and narrow junctions (arrow) are observed. Endoplasmic reticulum: ***er***. The bar indicates 600 µm

At the testis follicles body and efferent tubes can be distinguished Fig. 21. At each ovariole three regions are present: the terminal filament, the main body and a duct, the future pedicel. Fig. 22. The male gonads size is larger than the females at this development time.

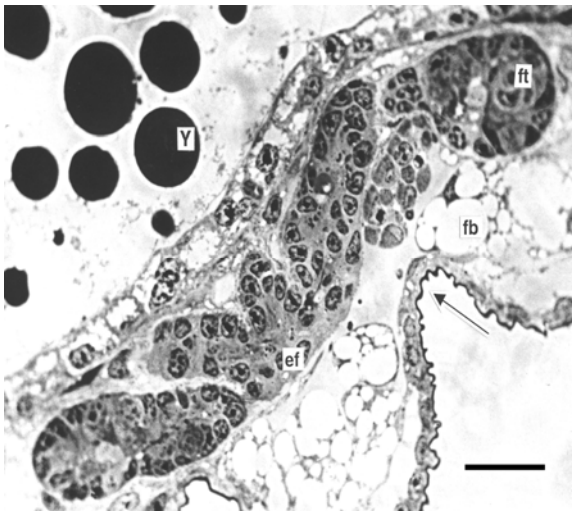

Fig 21. Histology section (2µm thick) showing the testis of a near to hatch embryo. The follicles region: ***ft,*** an efferent duct: ***ef,*** the fat body: ***fb,*** and the yolk: ***y.*** The arrow points the cuticle. The bar indicates 5,5µm.

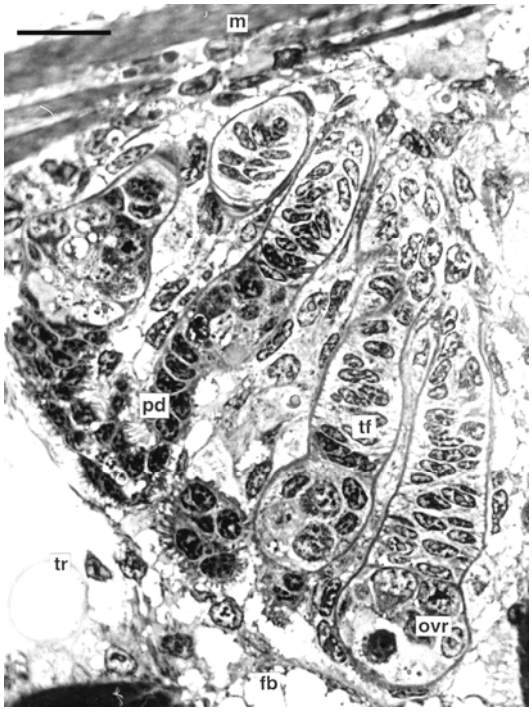

Fig 22. Female gonad histology section (2µm thick) from a tenth day after oviposition embryo. The structural units (ovarioles) already differentiated in three regions; filament: *tf*, ovarioles body: *ovr*, and pedicel: *pd.* Muscle: *m*, fat body: *fb*, trachea: *tr*. The bar indicates 25µm.

As the ultra-structure is concerned, germinal cell maintain their large nuclei and a conspicuous nucleoli, mitochondria in large number are present at the cytoplasm, they show a clear cut polarization Fig. 23. The relationship between these cells and the somatic is recognized as septata cell union type, some points show narrow unions Fig. 24. As development time goes on, the observed characteristics are very similar to the already described.

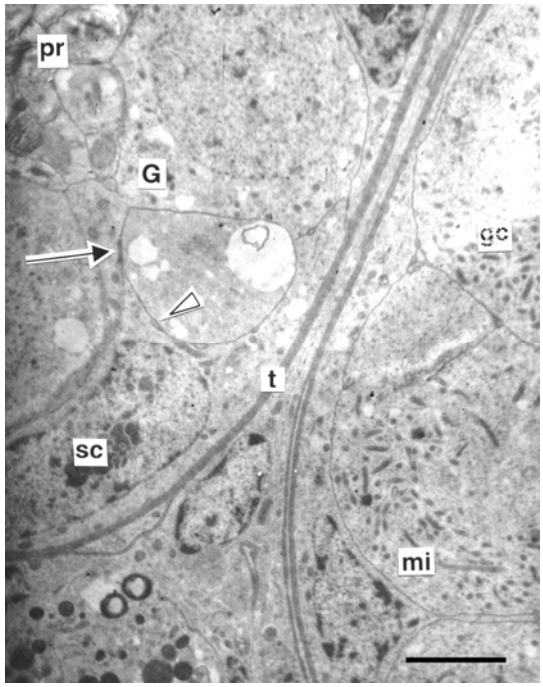

Fig. 23. Ovary thin section TEM micrograph from a just molted nymph. Two ovarioles has been sectioned each one surrounded by their specific tunica: ***t,*** and ovary stroma. Germinal cells: ***gc,*** show a rich mitochondria: ***mi,*** polarized population, and the presence of Golgi apparatus: ***G.*** Germinal cells are not in close contact one with the other, between them cytoplasmic processes: ***pr,*** are present. With these processes the germinal cells: ***gc*** are related by septata junctions (arrow) and gap junctions (arrow head); somatic cell: ***sc***. The bar indicates 2µm

All embryos at tenth day present perfectly established differences between both sexes. Other changes become evident at the present development phase, the secretion of the cuticle of the insect body, an increase of fat body size; of muscles both visceral and metameric related with body wall as well as the components of the respiratory system Fig. 25. Tenth day after oviposition up to first instar hatching, point a conspicuous size growth but any more of formation and differentiation.

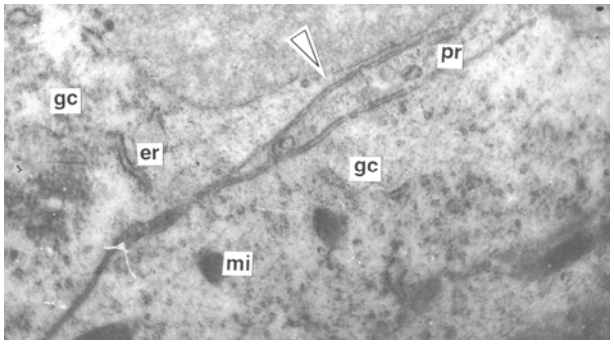

Fig. 24. Ovary thin section TEM micrograph from a just molted nymph, in which two germinal cells: ***gc,*** came in proximal neighborhood. The cytoplasm process: ***pr,*** between them show a septata cell junction type (arrow head). Mitochondria: ***mi***, endoplasmic reticulum: ***er***. The bar indicates 0,5µm

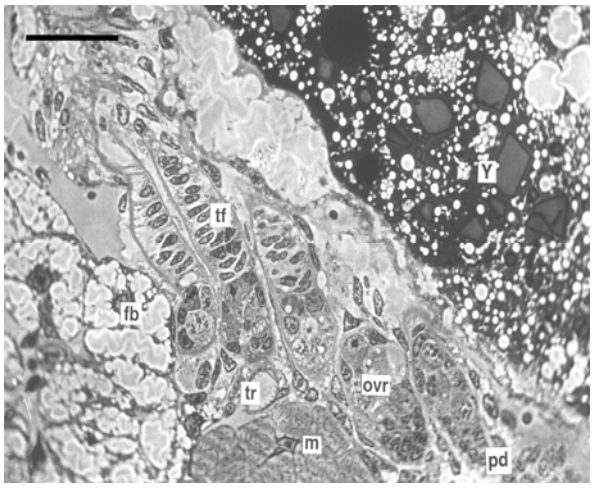

Fig 25. Ovary para-sagital (2µm thick) histology section from a ready to hatch embryo. Only five from the seven structural units have been taken in this slice. The section shows the ovariole regions: filament: ***tf,*** ovariole body: ***ovr,*** pedicel or duct: ***pd,*** fat body: ***fb,*** muscle: ***m,*** yolk:***y*** , trachea: ***tr.*** The bar indicates 20µm

At the end of embryo development as well as in just hatched nymphs, ovariole ultra-thin sections show under T.E.M. germinal cells which cytoplasm present a rich mitochondria population, throughout the cytoplasm, the connection between them is done by cytoplasm processes Fig. 26, within them septata joins and gap-junctions are present. These last two named cell unions type are also present to communicate germinal cells with somatic cells Fig. 27. At the center of the ovariol cytoplasmic processes are so numerous that labyrinths formation may appear.

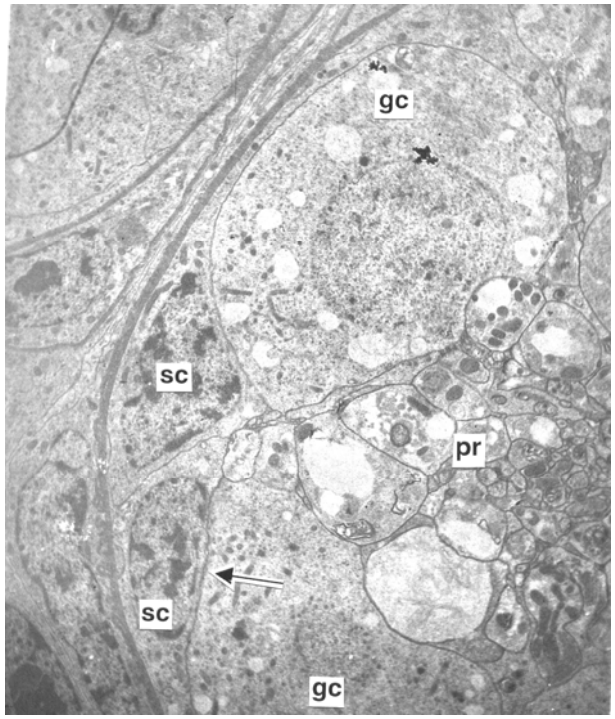

Fig. 26. Ovariole thin section TEM micrograph from a just hatched I (first) nymph. The germinal cells: ***gc,*** came not to be in close contact, a labyrinth maze like cytoplasmic processes: ***pr,*** are observed. They spread out all over the ovariole body. At the micrograph border somatic cells: ***sc,*** are present. Between them and the ***gc***, septata cell junctions can be found, (arrow). The bar, indicates 0,8µm.

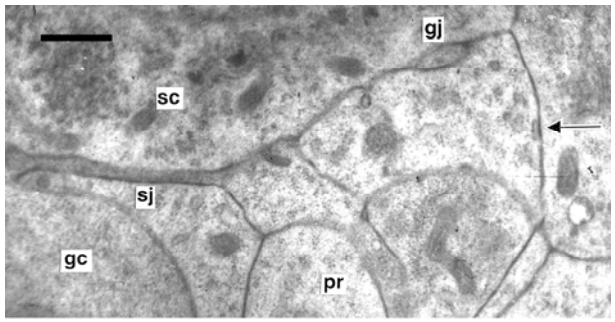

Fig. 27. Just hatched nymph I's ovariole TEM section micrograph**.** Several cell junction types between germinal cells: ***gc,*** somatic cells: ***sc,*** and the cytoplasm processes: ***pr***. Septata junction: ***sj,*** gap junction: ***gj,*** and a zone of narrow junctions are distinguished (arrow). The bar indicates 1,5µm.

Discussion

Triatoma infestans' gonads progress was analyzed from the start of embryo development both at histological and ultra structural levels. Insect's gonads development has antecedent for different species (7–9,13–14), the best description are three published papers for genus Drosophila, an holometabol diptera (15–17).The present work intent to increase gonadogenesis knowledge for *T. infestans,* adding new concepts to those present known for Hemiptera (18–21).

We have localized the primordial gonad between the third and fourth days after oviposition, when either completes eggs, light microscope 2µm thick and T.E.M ultrathin sections were analyzed. The careful examination on complete eggs allowed us to follow blastokinesis, movements that were taken as time parameter; considered by us a better time reference than number of days after oviposition alone. We have been able to follow general embryo morphology characteristics, and movements that change its position within egg envelopes by a 180° rotation around mid-transversal egg axis. Such a process takes place together with the gastrulation at early development time, between fourth and seventh days. It might have an important roll or be an adjuvant to gonadogenesis, since then the first steps in gonads morphogenesis take place (22). In fact the following steps occur:

- Coalescence of mesoderm precursors of the gonads.
- Cell dehiscence occurs, germinal cells, distinguished by their morphology, show a dynamic migration up to reach mesoderm somatic precursors.
- Gonads conformation is completed while blastokinesis is going on, so seven units are present when it concludes.

Taking into account what is stated at the former paragraph, 2µm thick sections were analyzed under the optical microscope, when the germinal band is localized in dorsal posterior position toward egg operculum, and the cephalic end is positioned toward the opposite egg end. The histology of blastoderm configuration together with the cell displacement was studied. Although mitotic figures are present in large number at early blastoderm manifestation that has been signaled as germinal cell for *Rohdnius* (18–20), our interpretation is that the whole "tissue" is rather a proliferating syncitium, from which only a group will be disengaged from the massive, differentiating later as germinal cells.

Three days after oviposition at abdominal metamers the first gonad sketches are localized, sketches that might be similar to the somatic mesoblast structure of such organ as is usually accepted for other species. Simultaneously actually we found that from the blastoderm cellular mass at the cephalic embryo zone the group of cells that detaches themselves, first in unordered way, but soon after forming lanes or chains that move in a cephalic – caudal direction a fact that can be compared to other insect species [14,23–25].

At fifth day after oviposition, such germinal cells are grouped near the gonad sketches, and coming apart of the group individually they start to enter the gonads in formation made mainly of somatic cells [26–28].

No analysis is made of the mechanisms that lead the behavior and migration of germinal cells, discussion and examination of this subject has been and is today the core of different hypothesis [29–32]. Germinal cells are recognized by their large nuclei, conspicuous nucleoli, ranges maintained all over their development and differentiation. While cells migrate they show a vacuolated cytoplasm that might be pointed to an accumulation product, perhaps of enzymatic character, a product that may be liberated to react with extra-cellular matrix allowing in part cells displacement as well as penetration across somatic cells of gonad primordial [28,33]. Cells boundary modification can be observed, changes from circular elongated contour defined by cytoplasmic projection, the so called "phyllopod", is taken as a sign of active participation during displacement and relocation.

Nevertheless migration movements may be stimulated by gonad primordia themselves giving rise to a polarization and oriented movements [34–36]. The influence of gene actions, gene message transcription on germinal cells behavior is the subject of nowadays studies, particularly in Drosophila [37–42].

At the same time five days post-oviposition, when germinal cells were already integrated to the somatic gonad, a follicle organization takes place. The number of seven follicles is fully established at the seventh day.

When blastokinesis is completed, about eighth day after oviposition, vitellus internalization takes place, the grater part is included in the digestive tube.

Except the food channel outermost (stomodaeum and proctodaeum), composed of ectoderm origin epithelium, the other part is of endoderm origin.

Between seventh and eighth day post-oviposition two follicle type structures can be distinguished, either somatic and germinal cells are intermingled without order or germinal cells appear to take a central position while somatic ones appear to positioned themselves around them. Our interpretation is that the prompt follicle organization correspond to the male gonad which is always larger than the female in early development [21].

Triatoma infestans gonads morphogenesis allowing the differentiation of testes follicles and ovarioles as well as their specific regions arises at early steps in embryo development.

Between ninth and tenth day both gonad types are fully developed, testes with their efferent duct that follow the follicles, and the seven ovarioles with a rather complex regionalization: **filament, body and pedicel,** the prominent one is the body where the germinal cells in bigger number are located.

The newly formed gonads ultra-structure shows that germinal cells are not apposed in close contact one with the other, between them cytoplasmic processes intercalate Septata type cell junctions are frequent, at possibility that their presence may facilitate the ensemble of new cells [43–45]. Germinal and somatic cells seam themselves by plane septata junctions, giving beside adhesion a selective permeable blending [46–52]. During all embryo development these two cell joint types are present.

The gonads differentiation is attained between ninth and tenth day, it is maintained without modifications up to hatching, 16 or 17 days after oviposition. Only growth takes place amid the last period of embryo development.

Acknowledgements

We acknowledge to Mr. Agustín V. Chertcoff for technical assistance.

References

[1] Wigglesworlh V.B. (1972) "*The Principles of Insect Physiology*" Seventh Edition. London. Chapman and Hall (Eds.), pp. 700-763.

[2] Gonçalves T.C.,Lent H.,Ribeiro de Almeida J.(1987) "Estudo anatómico e morfométrico dos folículos testiculares de algunas espécies de Triatominae (Hemíptera, Reduvidae). *Mem. Inst. Oswaldo Cruz.Río de Janeiro* 82(4):543-550.

[3] Barth R. (1973) "Estudos anatomicos e histologicos sobre a subfamilia Triatominae (Heteroptera, Reduvidae). Parte XXIII: O ovario de Triatoma infestans."*Memorias Do Instituto Oswaldo Cruz* 71(2)123-148.

[4] Mariano de Bozzini M.I., Ibáñez de Barrett C.I., Bozzini J.P. (1995) "New insight into Triatoma infestans'ovaries, Structure and Function."*Comunicaciones Biológicas* 13(1):59-70.

[5] Ibáñez de Barrett C.I., Bozzini J.P., Mariano de Bozzini M.I. (1999) "Cellular Interactions During The Female Gametogenesis of Triatoma infestans"(Klug,1834). *Biocell* 23(2):103-112.

[6] Mellamby H. (1935) " The early embryonic development of Rhodnius prolixus (Hemiptera Heteroptera) *Q. J. Microsc. Sci*. 78:71-90.

[7] Newcomer W.S. (1948) " Embriological development of the mouth parts and related structures of the milkweed bug Oncopeltus fasciatus (Dalla)" *J. Morphol*. 82:365-411.

[8] Anderson D. T. (1972) "*The development of hemimetabolous insects. Developmental Systems: Insects*." London, S.J. Counce and C.H. Waddington (eds) Academic Press 95-163.

[9] Gassner G., Sears S. (1976) "Preblastoderm nuclear division in the embryo of the large milkweed bug Oncopeltus Fasciatus (Dallas)" *Differentiation* 7:39-51.

[10] Fichera L.,Riarte A. (1992) "Embryology of Triatoma infestans (Klug) (Hemiptera Reduvidae) a Chagas disease vector. *Rev. Inst. Med. Trop. S. Paulo* 34(3):216-220.

[11] Mollenhauer H.H. (1964) "Plastic embedding mixtures for use in electrón microscopy" *Stain Technol*. 39:11.

[12] Reynolds E. S. (1963) "The use of lead citrate at high pH as an electron- opaque stain in electro microscopy" *J. Cell Biol*. 17:208-212.

[13] Miya K. (1984) "*Early embryogenesis of Bombyx Mori. Insect Ultrastructure*" New York In R.C. King and Akai H. (eds) Plenum Press Vol 2: 49-73.

[14] Zissler D. (1992) "From egg to pole cells:ultrastructural aspects of early cleavage and germ cell determination in insects" *Microsc. Res. Tech*. 22(1):49-74.

[15] Campos Ortega J. A., Hartenstein V. (1985) "*The Embryonic Development of Drosophila Melanogaster*" Berlin, Springer Verlag.

[16] Tanaka S. S., Toyooka Y., Akasu R., Katoh- Fukui Y., Nakahara Y., Suzuki R., Yokoyama M. , Noce T. (2001) "The mouse homolog of Drosophila Vasa is required for the development of male germ cells" *Genes Dev*. 14(7):841-853.

[17] Heller A., Steinmann-Zwicky M. (1998) "In Drosophila female gonadal cells repress male specif" *Develop*.73:203-209.

[18] Kelly G. M., Huebner E. "Experimental analysis of Rhodnius prolixus (Insecta, Hemiptera) embryogenesis (1986b).In H. C. Slaukin (ed), *Progress in Developmental Biology*. Part A. New York. Al R. Liss. Inc pp 423-426.

[19] Kelly G.M., Huebner E. (1989) "Embryonic Development of the Hemipteran Insect Rhodnius prolixus" *J of Morphol*. 199:175-196.

[20] Heming B. (1984) "*Insect embryogenesis and germ cells in Rhodnius prolixus*" Department of Biological Sciences, University of Alberta.

[21] Ibáñez de Barrett C.I., Bozzini J.P., Mariano de Bozzini M.I. (2004). "Differentiation and morphogenesis of Triatoma infestans (Klug,1834) Female gonads.I-Post embryonic development" *Biocell* 28(3):259-269.

[22] Leptin M. (2005) "Gastrulation Movements: the Logic and the Nuts and Bolts" *Developmental Cell* 8(3):305-320.

[23] Nievwkoop P. D., Sutasurya L. A. (1981) "*Primordial Germ Cells in the Invertebrates. From Epigenesis to Preformation*" Cambridge, Cambridge University Press.

[24] Subtelny S., Penkala J.E. (1984) "Experimental evidence for a morphogenic role in the emergence of primordial germ cells from the endoderm in Rana pipien. *Differentiation*. 26:211-219.

[25] Sonnenblick B. P. (1941) "Germ cell movements and sex differentiation of gonads in the Drosophila embryo" *Proc Nat. Acad. Sci. USA* 27:484-489.

[26] Gompters M., Garcìa Castro M., Wylie C., Heesman J. (1994). "Interactions between primordial germ cell play a rol in their migration in mouse embryos" *Development* 120:135-141.

[27] Jaglarz M. K., Howard K. R. (1994) "Primordial germ cell migration in Drosophila melanogaster is controlled by somatic tissue" *Development* 120:83-89.

[28] Jaglarz M. K.,Howard K.R. (1995) " The active migration of Drosophila primordial germ cells" *Develop*. 121:3495-3503.

[29] Warrior R. (1994) "Primordial germ cell migration and the assembly of the Drosophila embryonic gonad" *Dev. Biol*. (1):180-194.

[30] Mahowald A.P. (2001) "Assembly of the Drosophila germ plasm" *Int. Rev. Cytol.* 203:187-213.

[31] Chang C., Dearden P.,Akam M. (2002) "Germ line development in the grasshopper Schistocerca gregaria" *Developmental Biol.* 252(1):100-118.

[32] Roosen – Runge E. C.(1973) "Germinal cell loss in normal metazoan spermatogenesis" *J. Reprod.Fertil*. 35:339-348.

[33] Styhler S., Nakamura A., Swan A., Suter B., Lasko P. (1998) "Vasa is required for Gurken accumulation in the oocyte and is involved in oocyte differentiation and germline cyst development" *Int. Rev. Cytol*. 125:1569-1578.

[34] Reichman-Fried. M., Minina S., Raz E. (2004) "Autonomus modes of behavior in primordial germ cell migration*"* *Dev. Cell* 6(4):589-596.

[35] Clark I. B. N., Tarman A. P., Finnegan D. (2007) "Live imaging of Drosophila gonad formation reveals roles for Sx 4 in regulation germline and somatic cell migration" *Dev. Bio*. 7:52.

[36] Coffman C. R. (2003) "Cell migration and programmed cell death of Drosophila germ cells" *Ann. N Y Acad. Sci*. 995:117-126.

[37] Curtis D., Apfeld J., Lehman R. (1995) "Nanos is an evolutionarily conserved organizer of anterior-posterior polarity" *Development* 121(6):1899-1910.

[38] Kobayashi S., Yamada M.,Asaoka M., Kilamura T. (1996) "Essential role of the posterior morphogen nanos for germline development in Drosophila" *Int. Rev. Cytol*. 380:708-711.

[39] Kang D., Pilon M., Weisblat D. A. (2002) "Maternal and zigotic expression of a nanos- class -gene in the Leech Helobdella robusta: Primordial Germ Cells Arise from Segmentel Mesoderm" *Dev. Biol*. 245(1):28-41.

[40] Wang Z., Lin H. F. (2004) "Nanos maintains germline stem cell self renewal by preventing differentiation" *Science* 303(5666):2016-2019.

[41] Hayashi Y., Hayashi M., Kobayashi S. (2004) "Nanos suppresses somatic cell fate in Drosophila germline" *Proc. Nat. Acad. Sci. USA* 101(28):10338-10342.

[42] Gavis E. R., Chatterrjee S., Ford N. R., Wolff L. J. (2007) "Dispensability of nanos mRNA localization for abdominal patterning but not for germ cell development" *Mechanis. of Devel.* 125(1-2):81-90.

[43] Giorgi F., Postlethwait. (2005) "Development of gap junctions in normal and mutant ovaries of Drosophila melanogaster" *J. Morphol*. 185(1):115-129.

[44] Lane N. J., Skaer H. B. (1980) "Intercellular junction in insect tissues" *Adv. Insect Physiol.* 15:35-213.

[45] Szöllösi A., Marcaillou C. (1980) "Presence of gap junctions between germ and somatic cells in the testis of an insect, the moth Anagasta kuehniella" *Cell Tissue Res*. 213:137-147.

[46] Lawrence T. S., Beers W. H., Gilula N. B. (1978) "Transmission of hormonal stimulation by cell to cell comunication" *Nature. London* 272:501-505.

[47] Marcaillou C., Szöllösi A. (1975) "Variations de permeabilite du follicule testiculaire chez le criquet Locusta migratoria (Orthoptera) au cours du dernier stade larvaire" *C. R. Acad. Sci. Paris* 281:2001-2004.

[48] Noirot-Timoyhée C., Noirot C.(1980) "Septate and scalariform junctions in arthropods" *Int. Rev. Cytology* 63:97-140.

[49] Russell L. D. (1980) "Sertoli germ cell interrelations: a review" *Gamete Res*. 3:179-202.

[50] Wood R. L., Kuda A. M. (1980) "Formation of junctions in regenerating hydra: Septate junctions" *J. Ultrastruct. Res*. 70:104-117.

[51] Woodruff R. I. (1979) "Electronic junctions in Cecropia moth ovaries" *Develop. Biol*. 69:281-295.

[52] Zamboni L (1974) "Fine morphology of the follicle wall and follicle cell oocyte association" *Biol. Reprod.* 10:125-149.