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Comparative Results of Action of Natural and Synthetic Acaricides in Reproductive and Salivar Systems of *Rhipicephalus sanguineus* - Searching by a Sustainable Ticks Control

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1. Introduction

Ticks have been well studied and are one of the most important groups amongst the Arthropod, showing that there is of great interest in the medical and veterinarian aspects, mainly because of the fact that they appear to be carriers of many illnesses that attack domestic and wild animals, as well as humans (Sonenshine, 1991).
The ticks in rural or urban environment represent a big problem for society. For dairy producers and especially for meat and leather producer these ectoparasites cause huge financial losses due to their fixation in its hosts. Already in urban environments infestations in domestic dogs, as well as common contaminate the home environment, through the transmission of serious diseases to man.

2. *Rhipicephalus sanguineus* ticks

The importance of the ticks meanly *Rhipicephalus sanguineus* in the domestic world can be explained by the introduction of dogs in houses, for example, as companion animals consequently its ectoparasites facilitates the propagation of biopathogens those cause diseases both to the dogs and the human being; therefore these ticks are nowadays considered "urban plagues"
Numerous studies are currently under way to find an effective control strategy that would minimize the damage caused by these ectoparasites. A new tick control approach is an immunological one consisting in the identification, isolation and synthesis of proteins those protect tissues and organs of the tick, mainly those of the reproductive and glandular systems, with the aim of developing a vaccine (Tellam et al, 1992; Willadsen, 1997). However, nowadays the most efficient method to control tick populations is by suing chemical compounds, such as permethrin, fipronil and selamectin (active ingredients of different acaricides) which frequently act in the nervous system of the ticks (Mencke et al., 2003).

3. Ticks control

The challenge of controlling these arthropods has long been subject of several studies and the results obtained have been the formulation of new acaricides (synthetic chemicals) that are now widely used. However, collateral damages has been reported, including the emergence of individuals resistant to the drugs applied, which raises the source of new generations of ticks those are little affected by application of the acaricides. Also in this demand, the pharmaceutical industry (meanly of veterinary products) frequently releasing acaricides in an attempt to circumvent this problem. However, specific studies on the tick's cell biology are still scarce and little is known about the consequences of its utilization in non-target organisms.

3.1 Permethrin acaricide

The toxicity of an acaricide is defined as extent or degree to which a chemical substance to kill or injure the target organism. In this way, the toxicity of a drug is determined by running laboratorial tests on animals and it is expressed as LD_{50} (lethal dose fifty) and LC_{50} (lethal concentration fifty) values and are the amount or concentration, respectively, of the pesticide's active ingredient that is required to kill 50% of the tested animals under standardized tests conditions (Garcia-Garcia et al., 2005). The first study to described a detailed protocol of laboratorial procedures to determinate de LC_{50} of permethrin using semi-engorged females of *R. sanguineus* ticks was performed by Roma et al. (2010) who established the LC_{50}=2062 ppm (1.549-2.675). Acaricides currently available in the market have permethrin (chemical substance that cause a nervous impulses disorders and the ticks suffer excitement, indicated by tremors and spasms followed by paralysis and death) as active ingredient in concentration higher than 300.000 ppm and according Roma et al. (2010) permethrin in lower concentrations (approximately 100 times less) would be enough to kill *R. sanguineus* ticks, although this process would be slower.

3.2 Permethrin x *Rhipicephalus sanguineus* salivary gland

The feeding success of the ticks is the result of the action of the metabolism of the salivary glands, organs that are responsible for the fixation of the tick in the host (Moorhouse and Tatchell, 1966), by osmoregulation (Sonenshine, 1991), the inhibition of the host defense mechanisms such as coagulation and inflammation (Nuttal and Strickland, 1908: Paesen et al., 1999) and by the digestion of the tissues (Walker et al., 1985). Therefore, salivary glands are organs indispensable for the feeding process and therefore make ticks very biological successful organisms.

The tick's salivary glands are paired organs found in its celomatic cavity and contain approximately 1400 acini each (Walker et al., 1985). Many histological descriptions of salivary glands have been made in different species of ixodides ticks including by Furquim et al (2007, 2008). In summary, there are three different types of acini in females (I, II, III) and four in males (I, II, III and IV) and type I acini cells are agranular and have the osmoregulatory process as the main function, while types II, III and IV acini cells are granular and responsible for the other previously mentioned functions.

According to Nodari et al. (2011) the permethrin chemical induced morphophysiological changes in salivary glands of *R. sanguineus* semi-engorged females, even at lower concentrations, causing tissue changes compromising the organ metabolism, essential to the

feed process of the tick. The salivary glands in individuals subjected to 206 ppm of permetrhrin presented acini I morphologically altered (irregular shape and dilated lumen) corroborating Pereira et al. (2011), who reported that these acini in the same tick species were also affected by fipronil acaricide. These authors suggested that the acini I would be ormoregulators and through the saliva, they could remove the toxic compound from the hemolimph, since the lumen diameter of the acini suffer a significant increase. This authors showed that females subjected to permethrin at 2062 ppm presented only few acini I, with the other ones being classified as indeterminate, since they have lost their morphological and histological characteristics due the degeneration process by the toxic agent. The Nodari et al. (2011) results confirmed that permethrin, besides the proven neurotoxic action, also accelerates glandular tissue degeneration, an event that will occur naturally and with greater intensity only after full female engorgement (Fig. 1,2).

3.3 Fipronil x *Rhipicephalus sanguineus* salivary glands

In this sense, Oliveira et al. (2011) performed another study bringing information about the action of fipronil acaricide, using *R. sanguineus* as a biological model. Fipronil is a broad-spectrum chemical agent available in the market in a range of formulations from attractive baits for ant and cockroach control to sprays used in veterinary products for the control of ticks, fleas and mites (Penaliggon, 1997; Hugnef et al,. 1999; Higgins et al. 2001) and his effect are not fully known, as well as, the direct and indirect consequences of its use. The fipronil act mainly in the ticks GABA system, consisting of inhibitory neurotransmitters of neuromuscular junctions and central nervous system synapses (Denny, 2001). The authors developing an appropriate protocol for an in vitro bioassay (AIT) monitored daily. The LC_{50} and the 95% confidence interval were also determined. The increase in the concentration of fipronil (1ppm- 100 ppm) caused a progressive increase in the mortality of semi-engorged *R. sanguineus* females.

Pereira et al. (2011) in a study performed with the same chemical substance showed the action of fipronil in salivary glands of the same species females subjected to different concentrations. The acaricide accelerated the natural degeneration process of these glands, as well as reduced the number of the acini present in the gland as the concentration of the chemical compound is increased, making the organ unable to secrete its final saliva. From this process only the glandular ducts are preserved probably for being resistant to fipronil and having all the ducts (acinar, intermediate and common excretory ones) with lumen covered by cuticle, which prevented both the diffusion and the action of the acaricide on the epithelial cells. In the acini with intact cells the organelles were intact too (Fig. 3). In the altered cells there was evidence of apoptotic cell death which characteristic is to affect the cells individually and as synchronically. One common morphological characteristic of the cytoplasm of salivary gland of the *R. sanguineus* is the spherocrystal presence, constituted by phosphate and calcium carbonate and originated from the cisternae of the rough endoplasmic reticulum, however despite their presence in various systems of different species, no author stated what would be their real function (reserve of calcium, detoxication, ionic balance, etc.). In females subjected to 10 ppm of fipronil the glandular damages were extensive, considering that even the most resistant structure- the spherocrystals- became disorganized, resulting in a structure that went from perfectly round to completely irregular (Fig. 4, 5).

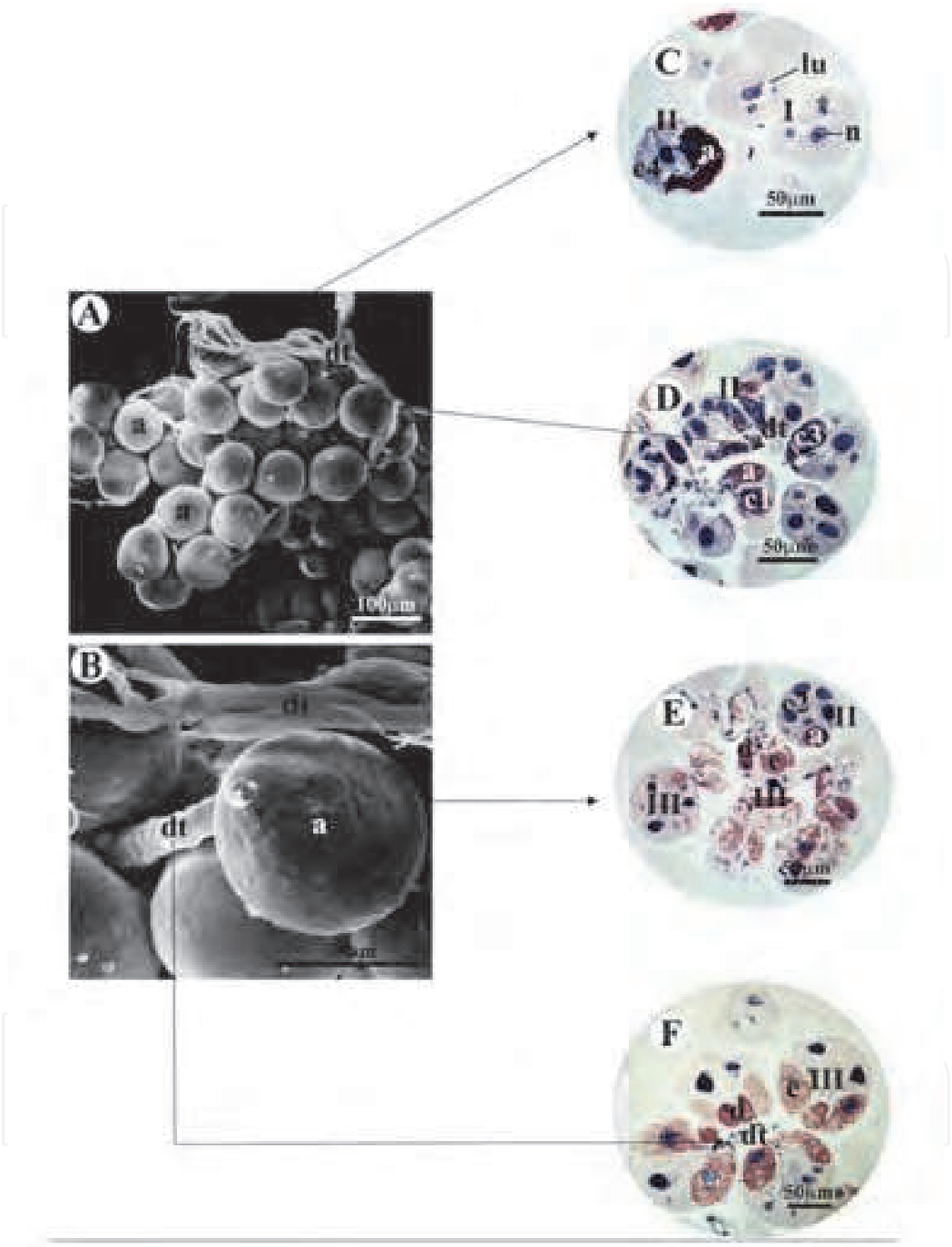


Fig. 1. (A-B) Scanning Electron Microscopy (SEM) of salivary glands of *Rhipicephalus sanguineus* semi-engorged female of the control group. (A) General view and (B) detail of the glandular acini (a) showing duct system (dt). (C-F) Histological sections of the *R. sanguineus* salivary glands of the control group stained with hematoxylin-eosin (HE) showing I (type I acinus), II (type II acinus) and III acini (type III acinus). dt = duct, n = nucleus of the central cell, * = nucleus of the peripheral cells, lu = lumen, a = a cell, c1 = c1 cell, c2 = c2 cell, c3 = c3 cell, c4 = c4 cell, d = d cell, e = e cell (Nodari et al, 2011).

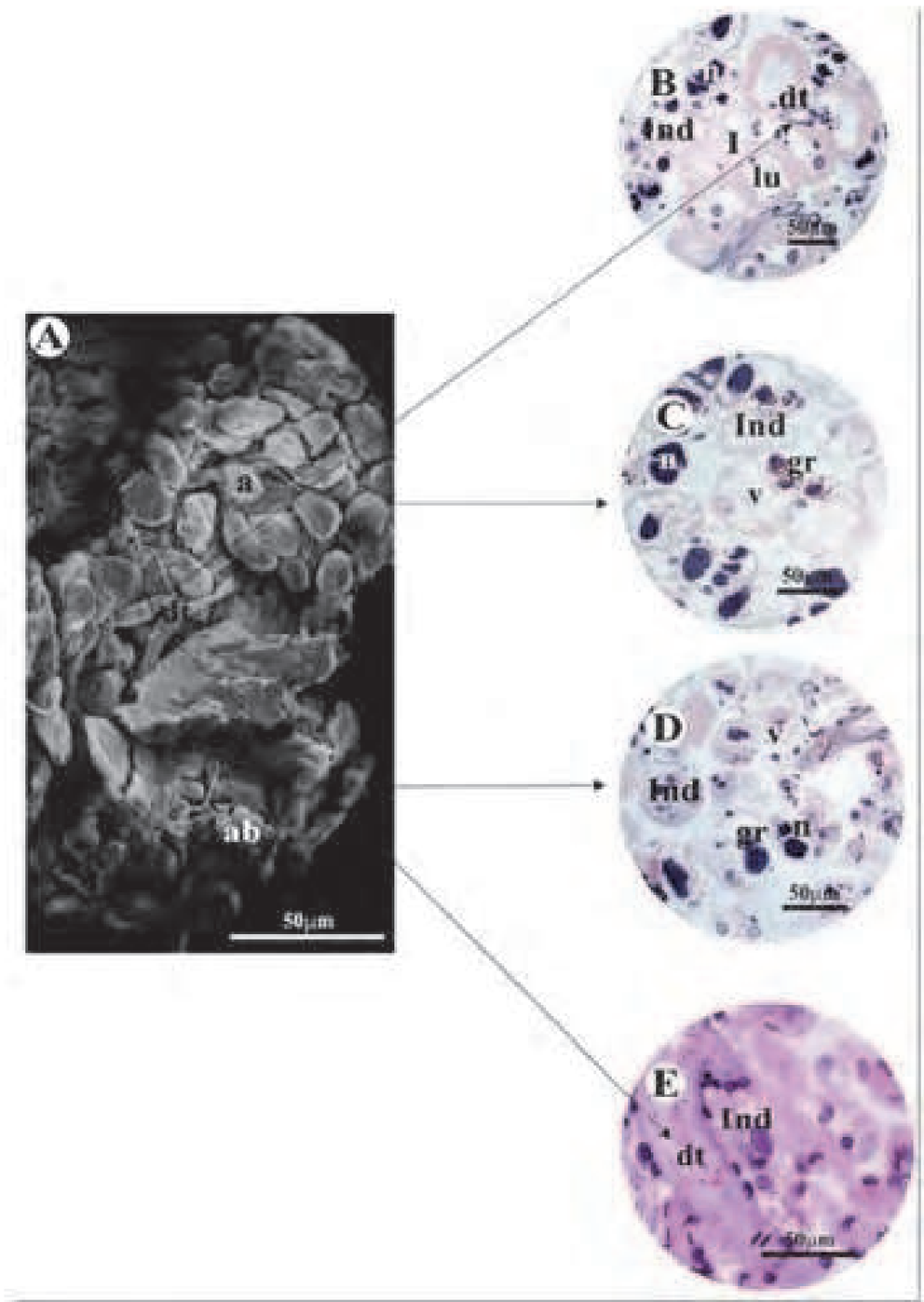


Fig. 2. (A) Scanning Electron Microscopy (SEM) of salivary glands of *Rhipicephalus sanguineus* semi-engorged females exposed to 2062 ppm of permethrin. (A) General view of the irregular acini (a), apoptotic body (ab) and ducts (dt). (B-E) Histological sections of the *R. sanguineus* salivary glands exposed to 2062 ppm of permethrin stained with hematoxylin-eosin (HE) showing I (type I acinus) with dilated lumen (lu), besides indeterminate acini (Ind). dt = duct, gr = granules, n = nucleus, v = vacuoles (Nodari et al, 2011).

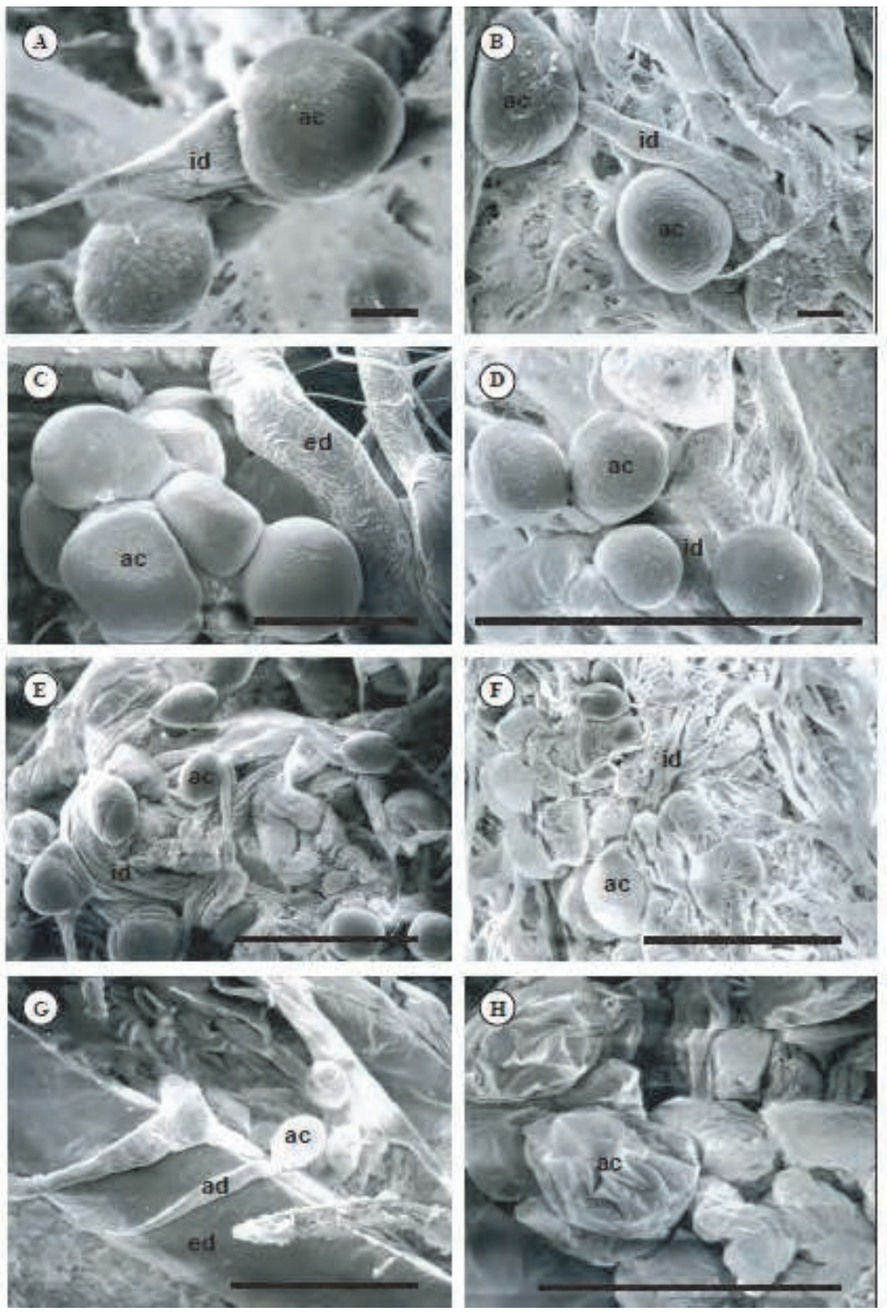


Fig. 3. Scanning Electron Micrographs (SEM) of semi-engorged females salivary glands of the tick *Rhipicephalus sanguineus* of the control group and treated with fipronil (1, 5 and 10ppm). A-B. Control group. Note in A and B, integral acini (ac) and intermediate ducts (id). C-D. Group subjected to the concentration of 1 ppm of fipronil. Less turgid acini (ac), common excretory duct (ed) and intermediate ducts (id). E-F. Group subjected to 5 ppm of fipronil. Less turgid acini (ac) and intermediate ducts (id). G-H. Group subjected to 10 ppm of fipronil. Acini (ac) with loss of turgidity or completely shriveled (shrunken) and irregular in shape, acinar duct (ad) and common excretory duct (ed) (Pereira et al, 2011). Bars: A-B: 10 µm; C-H: 100 µm.

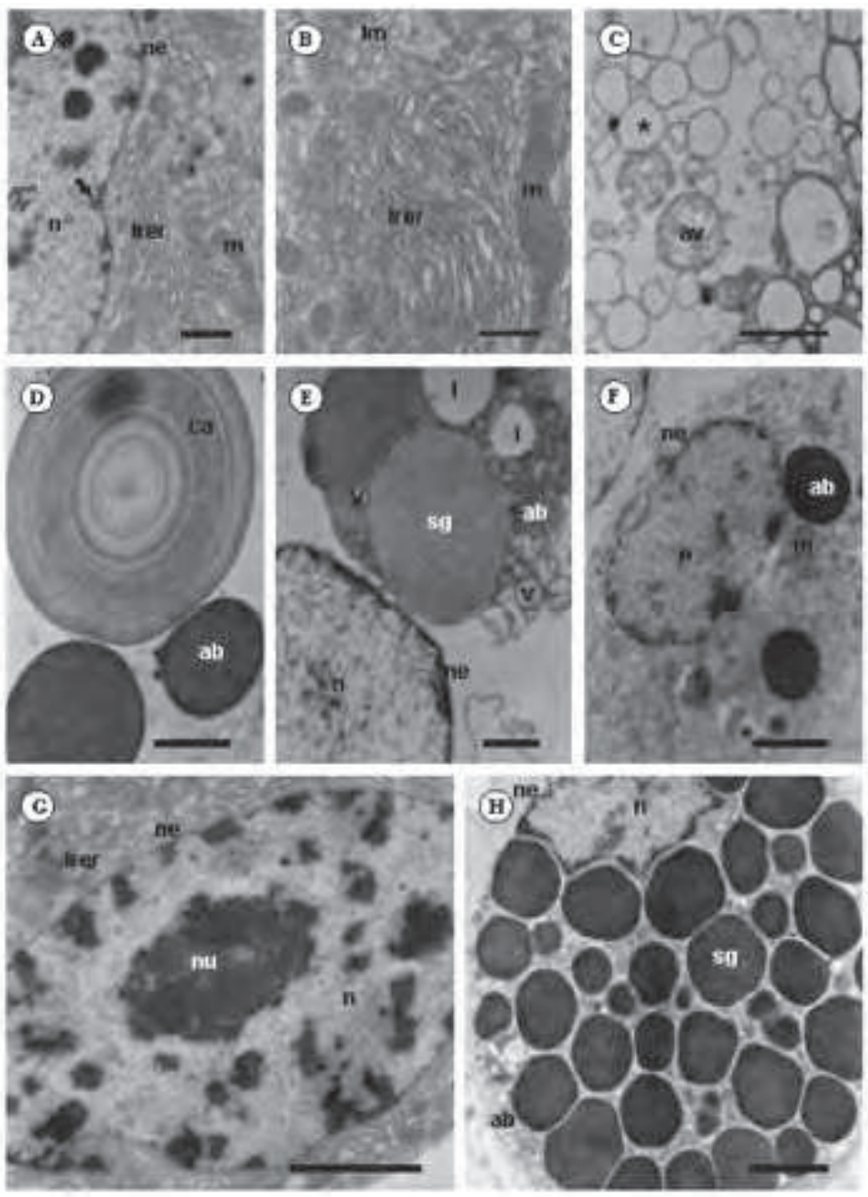


Fig. 4. Transmission Electron Micrographs (TEM) of semi-engorged females salivary glands of the tick *Rhipicephalus sanguineus* subjected to the concentration of 5 ppm of fipronil. A. Detail of the nuclei (n), marginalized chromatin (arrow) and of cytoplasm region of secretory cell with lamellar rough endoplasmic reticulum (lrer) and mitochondria (m). B. Detail of the membranous labyrinth (lm), lamellar rough endoplasmic reticulum (lrer) and mitochondria (m). C. Detail of the granular endoplasmic reticulum cisternae with dilatation (*) and autophagic vacuoles (av). D. Detail of the spherocrystals (ca) and small and homogeneous apoptotic bodies (ab). E-G. Detail of the nuclei cells (n) with nuclear envelope (ne) and marginalized chromatin. Apoptotic body (ab), lamellar rough endoplasmic reticulum (lrer), lipid (l), nucleolus (nu), proteic secretion granules (sg), vacuoles (v). H. Detail of the apoptotic body (ab) with nuclei cells (n), nuclear envelope (ne) and proteic secretion granules (sg) (Pereira et al, 2011). Bars: A, E-F = 1 μm; C-D, G-H = 2 μm; B = 10 μm.

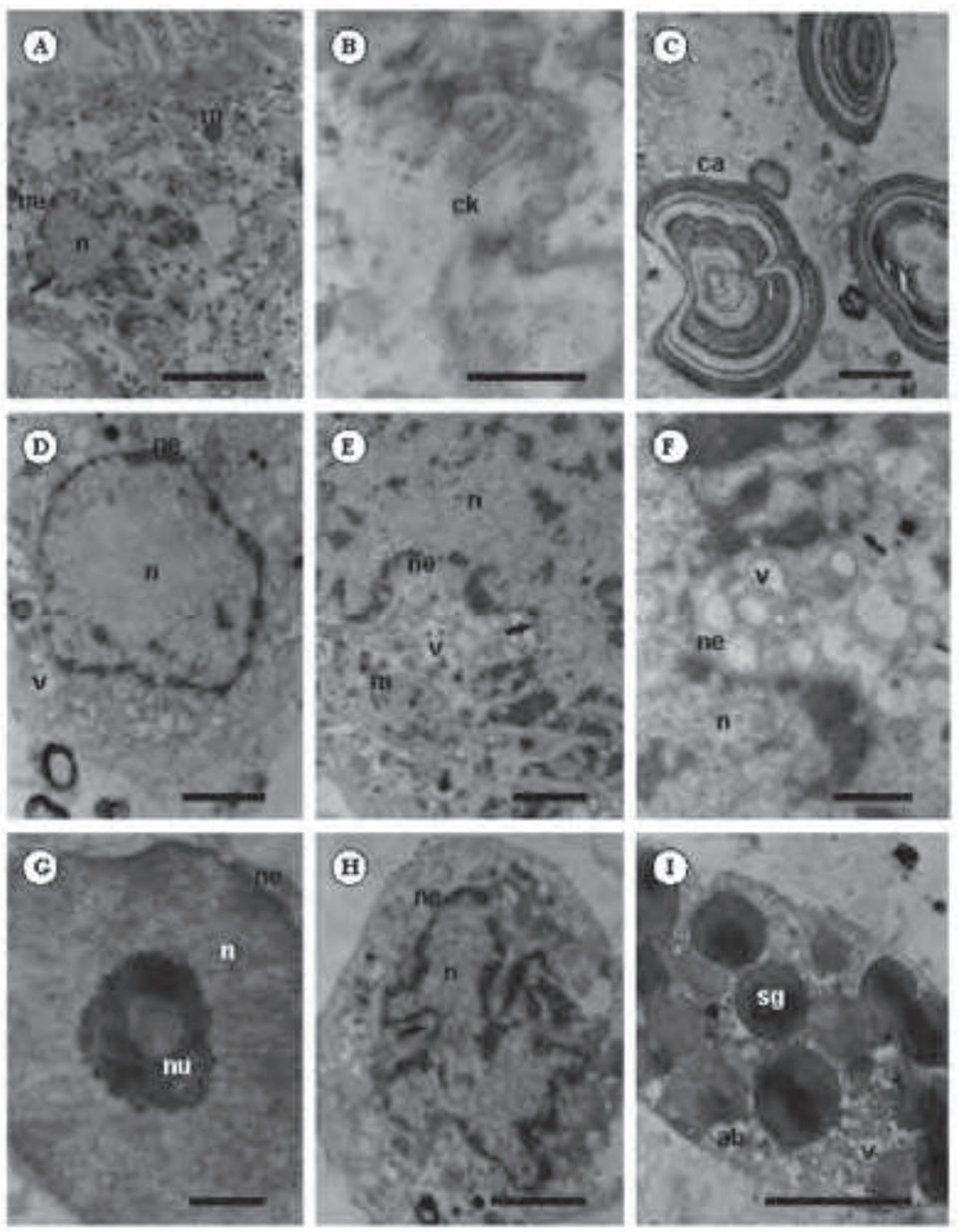


Fig. 5. Transmission Electron Micrographs (TEM) of semi-engorged females salivary glands of the tick *Rhipicephalus sanguineus* subjected to the concentration of 10 ppm of fipronil. A. Detail of completely disorganized cell where the irregular nuclei (n) with invaginations in the envelope nuclear (ne). B. Detail of the cytoplasm showing disarrange of the cytoskeleton (ck). C. Detail of the spherocrystals (ca) with completely irregular morphology. D-H. Detail of nuclei in process of cell death with invaginations (arrow) in the nuclear envelope (ne) and cytoplasmic vacuolation (v). Note in G, nuclei (n) with ring-shaped nucleolus (nu), typical characteristic of cells in cell death process. H. Apoptotic cell with irregular (arrow) nuclei (n). I. Detail of apoptotic body (ab) presenting extense process of vacuolation (v) and secretion granules (sg) (Pereira et al, 2011). Bars: B, F-G=1 μm; C-E, H = 2 μm; A, I = 5 μm.

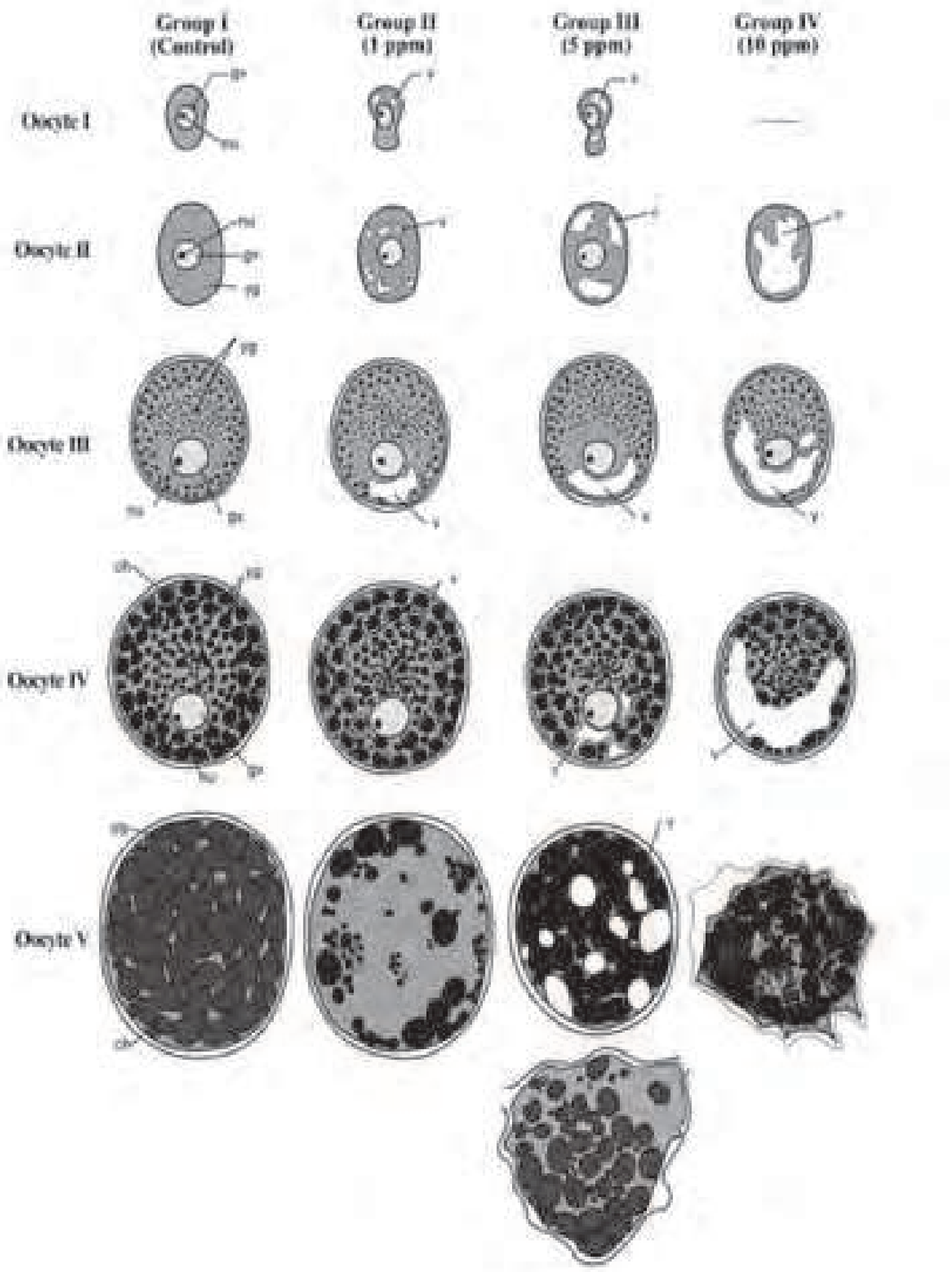


Fig. 6. Schematics drawing of the histological alterations observed in oocytes (I to V) of *Rhipicephalus sanguineus* treated with fipronil in different concentrations (control group, 1 ppm, 5 ppm, 10 ppm). ch= chorium; gv= germ vesicle; I= oocyte stage I; II= oocyte stage II; III= oocyte stage III; IV= oocyte stage IV; V= oocyte stage V; nu= nucleolus; pm = plasmic membrane; v=vacuoles; yg= yolk granules (Oliveira et al, 2008).

4. *Rhipicephalus sanguineus* ovaries

The tick ovaries in general are classified like a panoistic type; therefore it lacks nurse and follicular cells, as demonstrated in *Amblyomma cajennense* (Denardi et al, 2004), *R. sanguineus* (Oliveira et al, 2005) and *A. triste* females (Oliveira et al. 2006). It consists of a wall of epithelial cells, the pedicel, cellular structure that, besides supplying elements of the yolk to the oocytes, also connect them to the ovary wall (Till, 1961; Oliveira et al. 2005, 2006). The ovaries are usually filled with many oocytes, which will go through various developmental stages (I to V or VI) until they are ready for oviposition (Said, 1992, Saito el al. 2005).

4.1 Fipronil x *Rhipicephalus sanguineus* ovaries

Fipronil substance that acts inhibiting the development of ticks in pets animals (Taylor, 2001) and as a neurotoxic substance (Cole et al, 1993) also acts in the reproductive process of the ticks by altering both the structure and function of germinative cells of *R. sanguineus* (Oliveira et al., 2008, 2009). In these individuals ovaries the same authors detected alterations related with the size and morphology of the oocytes as well as presence of vacuoles in the cytoplasm and oocytes that had problem to succeeded in arriving at stage V (mature one), some of them had ruptured or withered, and had released their contents before the end of maturation. In females subjected to 10 ppm of fipronil (the maximal concentration tested) the oocytes in stage I were no longer observed, showing clearly that as the concentrations of fipronil increase the number and type of morphological changes in oocytes I increase as well (Fig. 6). Other data that would confirm the action of fipronil on the development of the *R. sanguineus* ovary would change the size of the oocytes, when comparing the control group to the three groups of treatment (1, 5 and 10 ppm of fipronil). The exposure of *R. sanguineus* feeding females to the fipronil leads to the susceptibility of its oocytes (in various degrees of development) to the chemical agent applied and, consequently, indicate the potential to cause damage in the reproductive process and to reduce the fertility of these ectoparasites (Fig. 7).

5. Natural acaricides

Thinking in the non-target organisms as well as in the environment preservation found alternative ticks control using biocompounds has an interesting subject with the intent to resolve some problems. In this sense is necessary to prevent the damages those are inflicted on non-target organisms, whereas the addition of financial savings the natural products could bring to producers and to the environment a sustainable management, since the environmental pollution caused by the indiscriminate use of toxic substances selects resistant animals, making the search for alternatives to natural and efficient chemicals, urgent. Plant compounds have been shown to be an important alternative for the control of these ectoparasites (Olivo et al., 2008).

5.1 Castor been oil esters x *Rhipicephalus sanguineus* salivary glands

Of the many plant species that have thus far been studied for future use in effective control of ticks is included the castor bean plant (*Ricinus communis*) which has recently emerged as a great promise, since their components such as oil extracted from it, is already widely known (biodiesel and dermatologic products). Also this plant has been supplying active principles that have enabled the fabrication of medical protheses, which besides being used in Brazil has been widely exported to the world.

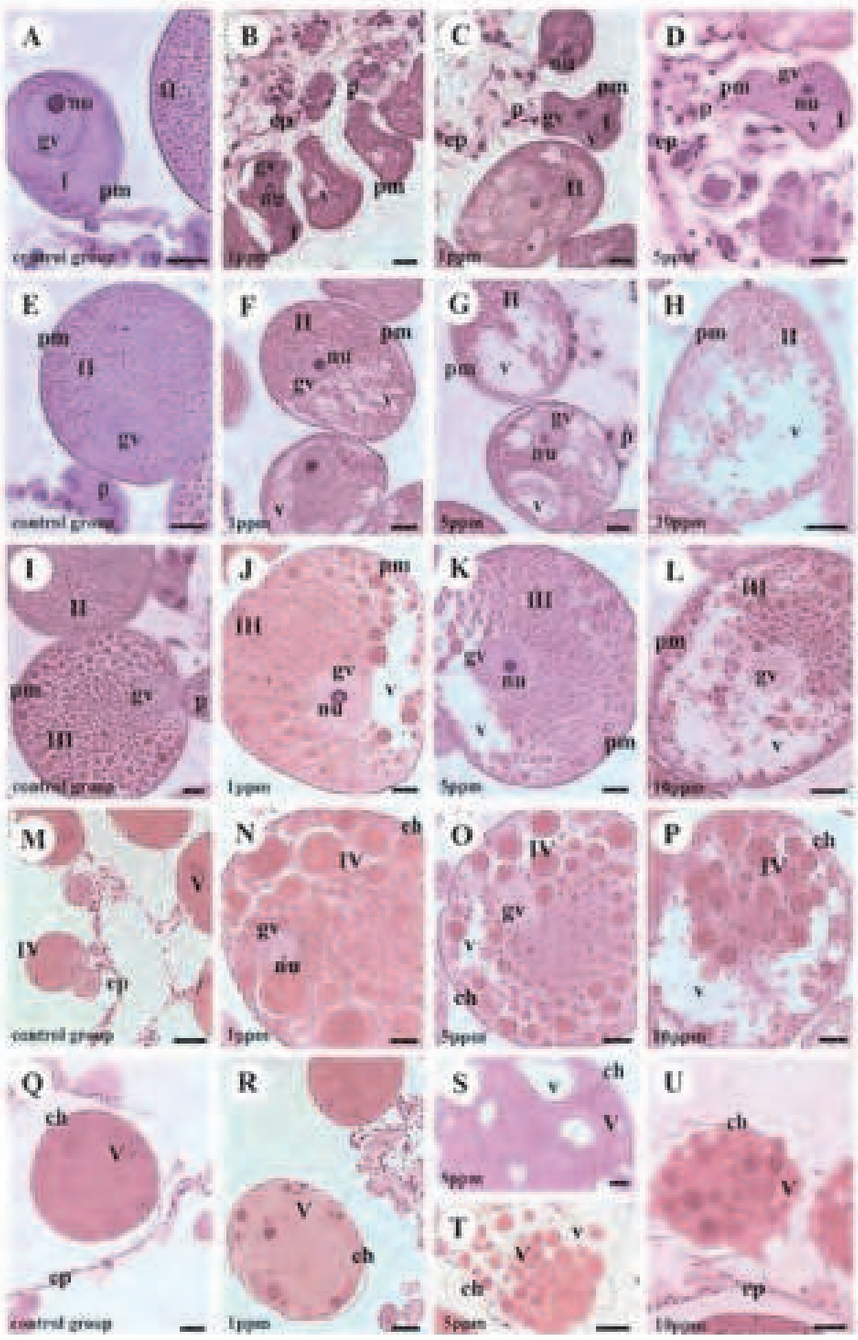


Fig. 7. Histological sections of *Rhipicephalus sanguineus* ovary stained by HE. A. E. I. M. O. Oocytes of the control group (group I) B. F. J. N. R. Oocytes of the group II (subjected to fipronil 1 ppm). C. G. K. O. S. T. Oocytes of the group III (subjected to 5 ppm). D. H. L. P. U. Oocytes of the group IV (exposed to 10 ppm). ch= chorium; ep= ovary epithelium; gv= germ vesicle; I= oocyte stage I; II= oocyte stage II; III= oocyte stage III; IV= oocyte stage IV; V= oocyte stage V; nu= nucleolus; p= pedicel; pm = plasmic membrane; v=vacuoles (Oliveira et al 2009). Bars: A-L= 0.02 mm; M= 0.1 mm; N= 0.02 mm; O= 0.02 mm; P= 0.02 mm; Q= 0.1 mm; R= 0.1 mm; S= 0.02 mm; T= 0.1 mm; U=0.05 mm.

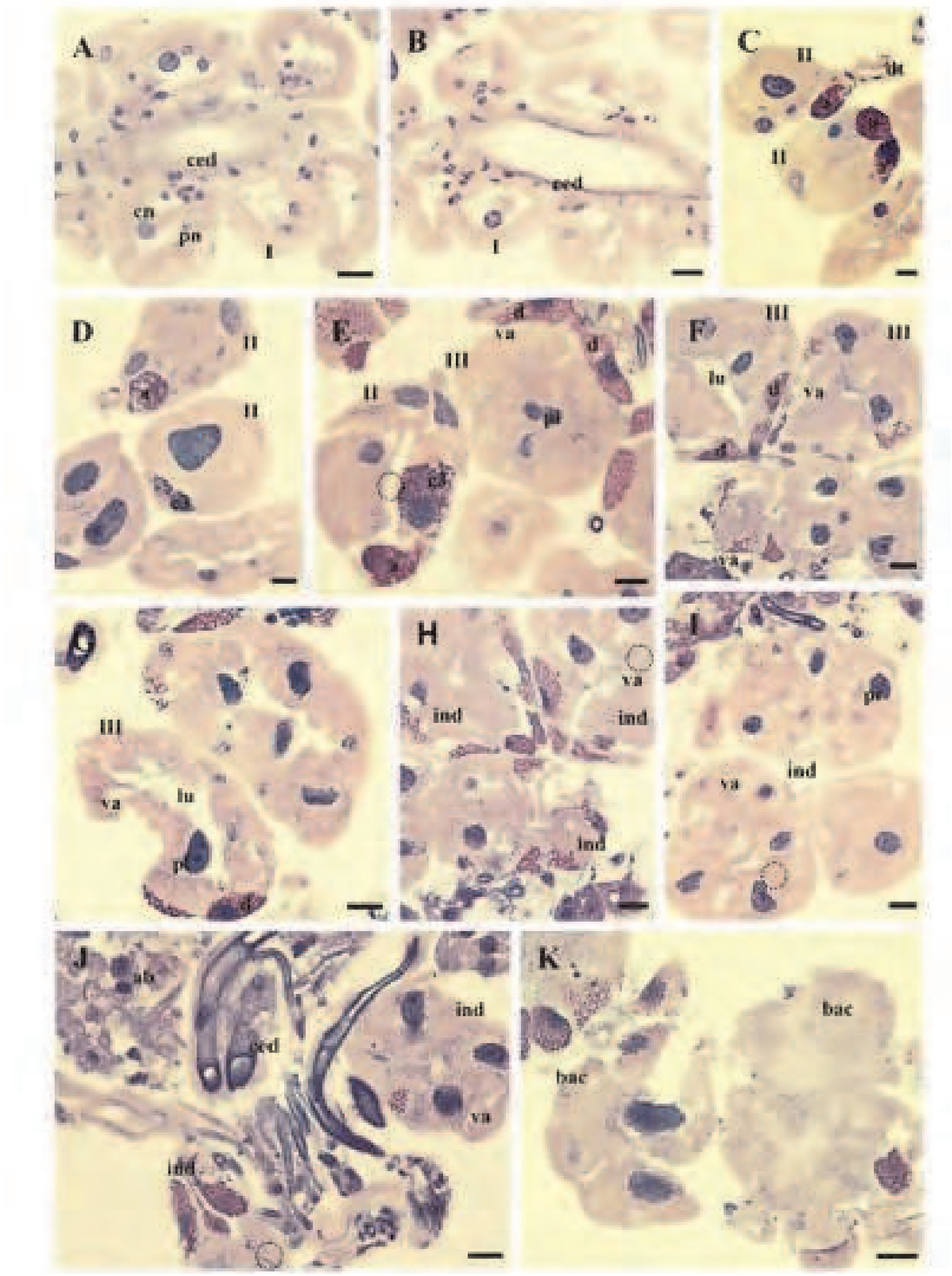


Fig. 8. Histological sections of salivary glands of engorged tick females *Rhipicephalus sanguineus* ticks (T1) stained with HE. I = acini I; II = acini II; III = acini III; ind = Indeterminate acini; a = cell a; c3 = c3 cell; d = d cell; bac = broken acini; dt = acinar duct (Arnosti et al. 2010) Bar: A–H = 20 um.

Recently a new application for the components extracted of castor plants *R. communis* has emerged from studies conducted by BCSTM (Brazilian Center of Studies on Ticks Morphology) headquartered at UNESP, Rio Claro, SP, Brazil coordinated by Profa. Dra. Maria Izabel Camargo Mathias in an attempt to offer an alternative way for tick control.

Ricinoleic acid esters from castor oil *R. communis* have been shown to efficiently in control micro and ectoparasites. Recent studies on the use of non-polluting plant the esters have shown that they act in the hydrolysis of saccharides and the dissolution of lipids in different biological systems (Mandelbaum et al., 2003). The consumption of castor oil esters by rabbits hosting the dog tick *R. sanguineus* significantly affected the vitellogenesis of the female, by making the oocytes non-viable and/or causing their death, and thus preventing new offspring from developing.

The action of the ester in salivary glands was also studied by Arnosti et al (2010a), and they showed that these esters, provided through food (feed+esters) for the rabbits hosts, enhancing and accelerated their degenerative process, that would otherwise occur slowly. It was also shown that the damage caused by esters in glandular cells was proportional to the concentration of the product, in other words at higher concentration (5g of ester (stabilized in NaCl)/Kg of commercial food for a period of 10 days. The results showed that, unlike conventional methods of chemical synthetic control, which kills ticks by neurotoxic action, different concentration of ester of *R. communis* oil did not cause the death of the ticks, but interfered in two interdependent systems, which are of paramount importance to these ectoparasites (by inducing premature degeneration of the salivary glands), and also acting in the reproductive process (preventing the vitellogenesis) (Fig. 8). Thus the action of esters on the salivary glands makes the ticks feeding process deficient causing the absence of some components in the hemolymph and consequently impairing the vitellogenic process of the oocytes (Arnosti et al., 2010a,b).

These first results performed in laboratorial assays by Arnosti et al (2010 a, b) were very promising showing the effect of this component not only in salivary glands but on the reproductive system of ticks *R. sanguineus* (reducing significantly the eggs viability), avoiding the individuals from completing the blood meal process considered necessary for effective ticks success (Fig. 9).

5.2 *Azadirachta indica* (neem plant) x *Rhipicephalus sanguineus* ovaries

In this sense, another plant of the Meliacea family (*Azadirachta indica*) or "neem" plant, have supplied actives products (extracted from leafs and seeds), which are effective to cause the reproduction damage in the ticks, by avoiding the maturation of oocytes causing consequently inhibition of the proliferation of ticks new generations. According by Denardi et al (2010) aqueous extracts obtained from leafs of neem, caused serious damages in the ovary cells of *R. sanguineus* engorged females, including morphological irregularities in the oocytes shape, changes in the yolk granulation and modifications in the germinal vesicle (oocytes nucleus) which contain the genetic material and consequently inhibing the progress of vitellogenesis, important process to reproductive success of this species (Fig. 10, 11).

Azadirachta indica would be an interesting alternative way to control ticks, since the azadiractin the active compound, can be easily obtained by management of the leafs to produce the extracts (aqueous and alcoholic), as well as saving non targets organisms once this plant is also consumed by humans in several products (like tea) in several countries in the world, meanly by Indian people.

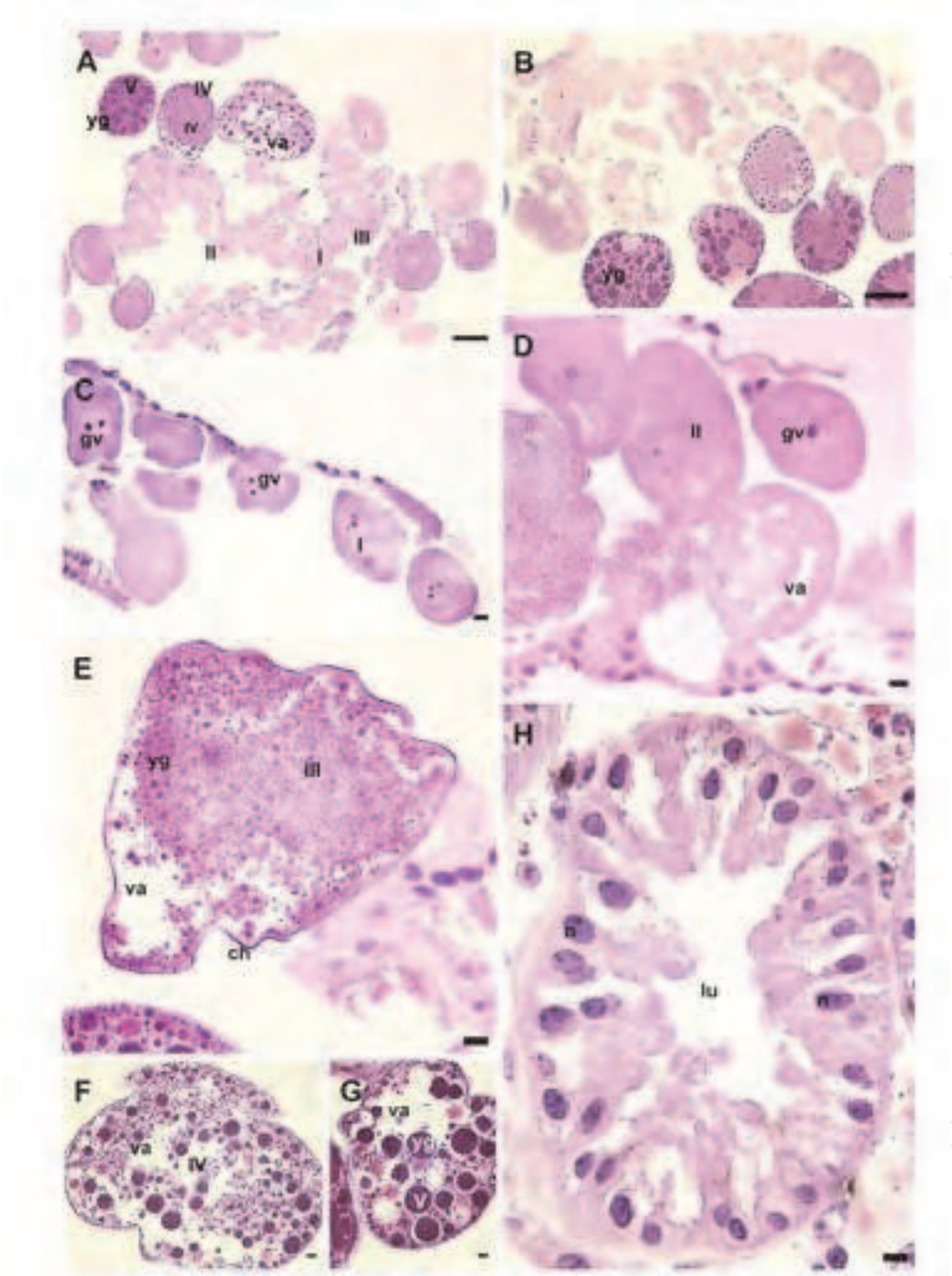


Fig. 9. Histological sections of ovary of *Rhipicephalus sanguineus* stained with HE. Treatment I with esters of *Ricinus communis* (T25g/kg food) (AB) Oocytes in various stages of maturation; vacuoles = va, yolk granules = yg; (C) Detail of oocyte I, germ vesicle = gv; (D) Details of oocytes II and III, germ vesicle = gv, vacuoles = va; (E) Detail of oocyte III, chorion = ch, vacuoles = va, yolk granules = yg; (F) Details of oocyte IV, vacuoles = va (G) Details of oocyte V, vacuoles = va, yolk granules = yg; (H) Detail of the ampole with sperm in the lumen= lu and nucleus of the ampole cell =n (Arnosti et al, 2010) Bar: A, B = 100 µm, Bar: C-H = 20 µm.

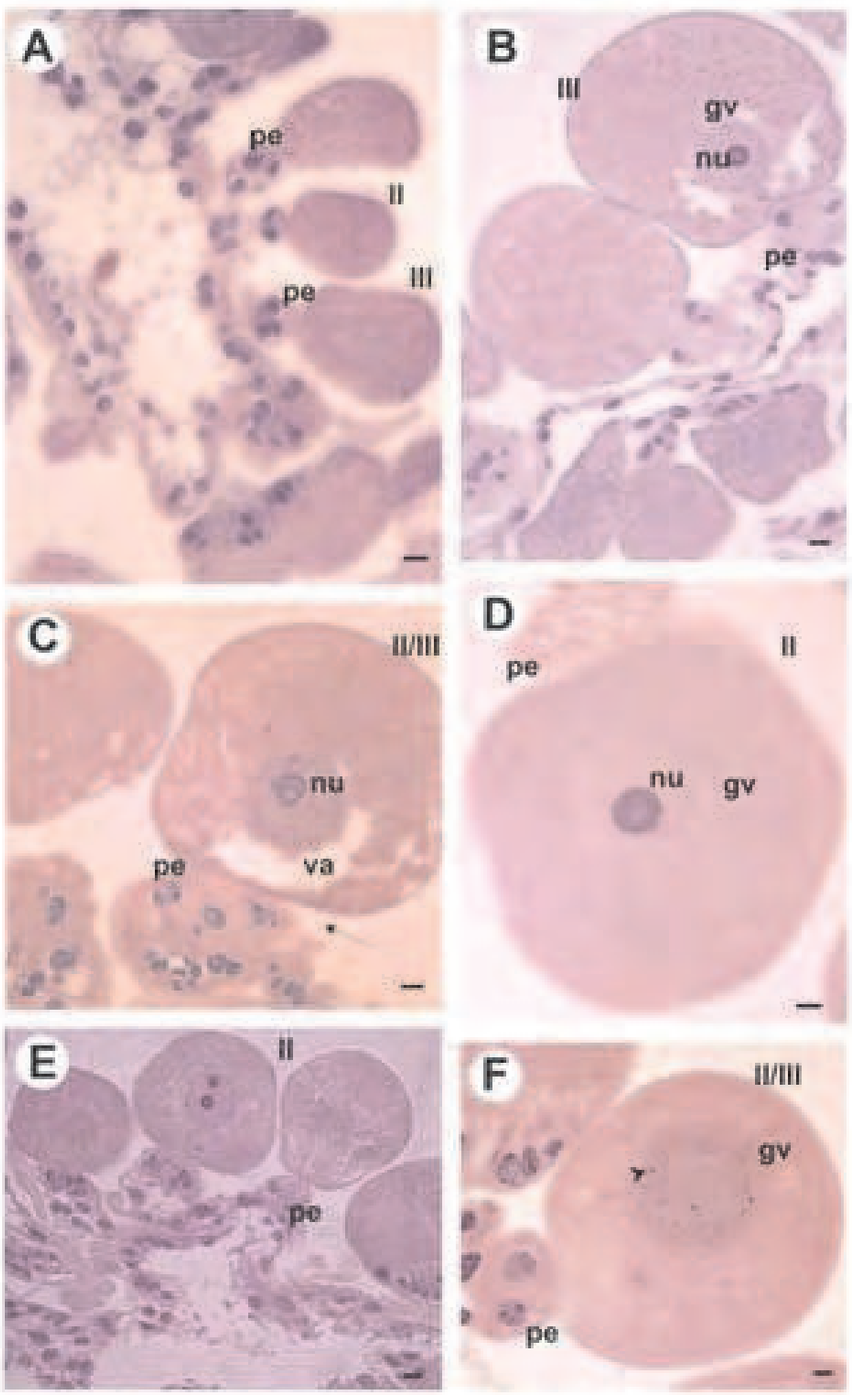


Fig. 10. Histological sections showing details of oocytes I and II, as well as the pedicel cells (pe), in the ovaries of semi-engorged females of *Rhipicephalus sanguineus* (A, C, E) and engorged ones (B, D, F) of *R. sanguineus* stained with hematoxilin and eosin (HE) (Denardi et al, 2010). Arrow = oocytes pole in contact with the pedicel cells (pe) where bigger vacuolation zone (va) can be observed. Note germinative vesicle (gv) with fragments of nucleoli (nu). Bar: 10µm A, B = control group; C, D = 10% treatment; E, F = 20% treatment.

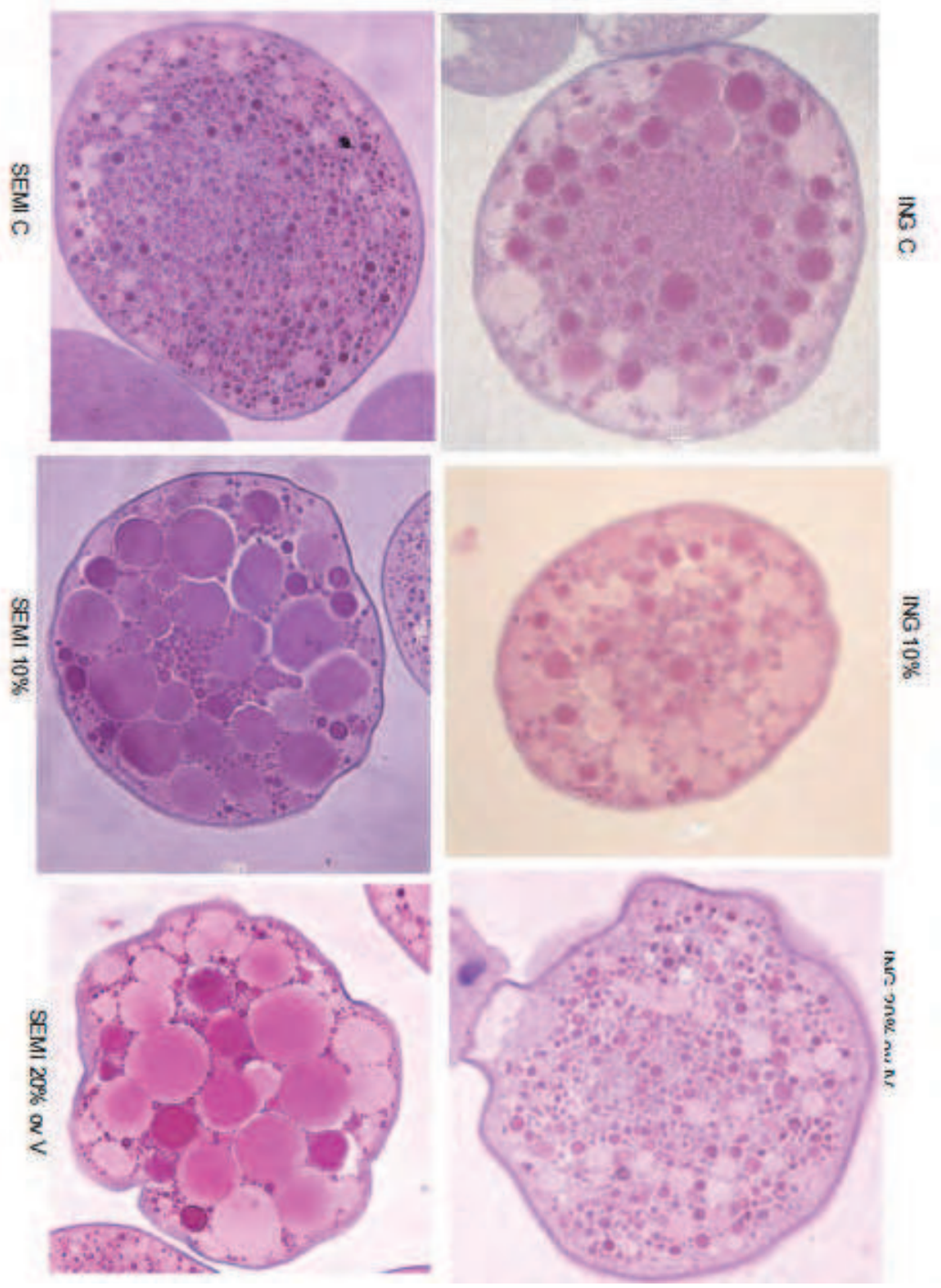


Fig. 11. Histological sections showing oocytes subjected to neem extract, in stages IV and V in the ovaries of *Rhipicephalus sanguineus* semi-engorged females (A, C, E) and engorged ones (B, D, F) stained with hematoxilin and eosin (HE), showing deformations (folds) (arrow) in the chorion (ch), and modified yolk granulation, when compared to the control group. Bar = 10µm (Denardi et al, 2010). A, B = control group; C, D = 10% treatment 10%; E, F = 20% treatment.

Besides these alternative option to control the ticks the BCSTM researcher in Brazil showed the effects of synthetic chemicals acaricides (permethrin and fipronil) in reproductive and salivary systems of *R. sanguineus* and demonstrate that lower doses and concentrations of acaricides would be used to prevent the ticks reproduction and feed, minimizing the damages to non-target individuals as well as to the environment (Roma et al., 2010, Oliveira et al. 2010).

The results presented here have also shown that doses of synthetic acaricides would be still efficient and much smaller and much less harmful to non-target organisms and to the environment being enough to reduce the harm caused by these ticks. This study confirm that chemical agents act by reducing and/or preventing the reproduction of females of *R. sanguineus* ticks, since many of the oocytes from individuals subjected to different acaricides exhibited major changes in the germ cells in relation to the control group.

Besides this, the natural compounds can be used in the management and combating of ticks within a context of sustainability of the systems, resulting in reduction of damage both to the hosts and environment (alternative tick control).

6. References

Arnosti, A., Brienza, P.D., Furquim, K.C.S., Chierice, G.O., Claro-Neto, S. Bechara, G.H., Sampieri, B.R.,. Camargo-Mathias, M. I. (2010a) Effects of *Ricinus communis* oil esters on salivary glands of *Rhipicephalus sanguineus* (Latreille, 1806) (Acari:Ixodidae). *Experimental Parasitology*, (October, 2010) D.O.I. 10.1016/j.exppara.2010.10.009, 2010ISSN: 0014-4894

Arnosti, A., Brienza, P.D., Furquim, K.C.S., Chierice, G.O., Claro-Neto, S. Bechara, G.H., Sampieri, B.R., Camargo-Mathias, M.I. (2010b). Effects of ricinoleic acid esters on the vitellogenesis of *Rhipicephalus sanguineus* (Latreille, 1806) (Acari:Ixodidae) tick. *Experimental Parasitology*, (October, 2010) D.O.I. ISSN: 0014-4894

Cole, L.M., Nicholson, R.A., Casida, J.E. 1993. Action of phenylpyrazole insecticides at the GABA-gated chloride channel. *Pesticide and Biochemistry Physiology*, 46-47-54. ISSN: 0048-3575

Denardi S.E., Bechara G.H., Oliveira P.R., Nunes, E.T., Saito, K.C., Camargo-Mathias, M.I. (2004) Morphological characterization of the ovary and vitellogenesis dynamics in the tick *Amblyomma cajennense* (Acari: Ixodidae). *Veterinary Parasitology* (July, 2004) 125:379-395, DOI:10.1016/j.exppara.2010.05.017. ISSN: 0304-4017

Denardi S.E., Bechara G.H., Oliveira P.R., Camargo-Mathias, M.I. (2010) *Azadirachta indica* A. Juss (neem) induced morphological changes on oocytes of *Rhipicephalus sanguineus* (Latreille, 1806) (Acari: Ixodidae) tick females. *Experimental Parasitology* (November, 2010) DOI:10.1016/j.exppara.2010.05.017. IssnISSN: 0014-4894

Denny, D.J.B. 2001. Efficacy of fipronil against ticks. *Veterinary Recherche* 148-124

Furquim, K.C.S., Bechara, G.H., Camargo-Mathias, M.I. 2007. Cytoplasmatic RNA and nuclear changes detected cytochemically during the degeneration of salivar glands of the tick *Rhipicephalus sanguineus* (Latreille, 1806) (Acari:Ixodidae*) Micron* (November, 2010) 39:960-966. ISSN: 0968-4328.

Furquim, K.C.S., Bechara, G.H., Camargo-Mathias, M.I. 2008. Death by apoptosis in salivary glands of the tick *Rhipicephalus sanguineus* (Latreille, 1806) (Acari:Ixodidae). *Experimental Parasitology* (February, 2008) 39:960-966.ISSN 0014-4894

Garcia-Garcia, E., Bussacos, M.A., Gisher, F.M. 2005. Impacto da legislação no registro de agrotóxicos de maior toxicidade no Brasil. *Revista de Saúde Pública* 39:832-839. ISSN 0034-8910.

Higgins, R.A., Sloderbeck, P.E. Brooks, H.L. 2001. *Field corns insect management for 2001*. Kansas: Kansas State University, 18-24.

Hugnet, C., Cadore, J.L., Bourdoiseau, G. 1999. Use of fipronil spray (0,25%) for the treatment of *Damlinia equi (Wereckiella equi)* infestation. *Pratique Veterinaire Equine* 31:65-68. ISSN:0395-7500.

Mandelbaunm, S.H., Santos, E.P., Mandelbaum, M.H.S. 2003. Cicatrização: conceitos atuais e recursos auxiliares- Parte II. *Anais Brasileiros de Dermatologia* 78:525-542. ISSN 0365-0596.

Mencke, N., Volp, P., Volfova, V., Stanneck, d. 2003. Reppelent efficacy of a combination containing imidaclorprid and permethrin against sand flies (*Phlebotomus papatasi*) on dogs. *Parasitology Research* 90:108-111. ISSN: 1432-1955.

Moorhouse, D.E., Tatchell, R. 1966. The feeding processes of the cattle tick *Boophilus microplus* (Canestrini): a study in host-parasite relations. Part I. Attachment to the host. *Parasitology* 56:623-632. ISSN: 0031-1820 EISSN: 1469-8161.

Nodari, E.F., Roma, G.C., Furquim, K.C.S., Bechara, G.H., Camargo-Mathias, M.I. 2001. Cytotoxic effects of permethrin in salivary glands of *Rhipicephalus sanguineus* (Latreille, 1806) (Acari:Ixodidade) semi-engorged females. *Experimental Parasitology* (February, 2011) DOI 10.1016/j.exppara.2011.02.003. ISSN: 0014-4894

Nuttal, G.H.F, Strickland, C. 1908. On the presence of an anticoagulin in the salivary glands and intestines of *Argas persicus. Parasitology* 1:300-310. ISSN: 0031-1820 EISSN: 1469-8161.

Oliveira P.R., Bechara G.H., Denardi S.E., Nunes E.T., Camargo-Mathias, M.I. (2005) Morphological characterization of the ovary and oocytes vitellogenesis of the ticks *Rhipicephalus sanguineus* (Latreille, 1806) (Acari: Ixodidae). *Experimental Parasitology* (March, 2005) 110: 146-156. . ISSN: 0014-4894

Oliveira P.R., Camargo-Mathias, M.I.,Bechara G.H. (2006) *Amblyomma triste* (Koch, 1844) (Acari: Ixodidae): Morphological description of te ovary and of vitellogenesis. *Experimental Parasitology* (March, 2006) 113: 179-185. .ISSN: 0014-4894

Oliveira P.R., Bechara G.H, Camargo-Mathias M.I. (2008) Evaluation of cytotoxic effects of fipronil on ovaries of semi-engorged *Rhipicephalus sanguineus* (Latreille, 1806) (Acari: Ixodidae) tick female. *Food and Chemical Toxicology* (March, 2008) 46:2459-2465. . ISSN: 0278-6915

Oliveira P.R., Bechara G.H, Camargo-Mathias M.I. (2009) Action of the chemical agent fipronil on the reproductive process of semi-engorged females of the tick *Rhipicephalus sanguineus* (Latreille, 1806) (Acari: Ixodidae). Ultrastructural evaluation of ovary cells. *Food and Chemical Toxicology* (February, 2009) 47:1255-1264. ISSN: 0278-6915.

Oliveira P.R., Bechara G.H, Denardi, S.E., Oliveira, R.J., Camargo-Mathias M.I. (2010) Citotoxicity of fipronil in mouse liver cells. *Veterinary Research* (February, 2009) DOI 10.1016/j.fct.2009.02.019. ISSN (Electronic Edition): 1297-9716

Oliveira P.R., Bechara G.H, Denardi, S.E., Pizano, M.A., Camargo-Mathias M.I. (2011) Toxicity effect of acaricide fipronil in semi-engorged females of the tick *Rhipicephalus sanguineus* (Latreille, 1806) (Acari: Ixodidae). Preliminary determination of the minimum lethal concentration and LC50. *Experimental Parasitology* (November, 2010) 127:418-422. ISSN: 0014-4894

Olivo, C.J., Madruga de Carvalho, N., Sousa da Silva, J.H., Flores Vogel, F., Massariol, P., Meinerz, G., Agnolin, C., Farias Morel, A., Volnei Viau, L. 2008. Óleo de citronela no controle de carrapatos de bovinos. *Ciencia Rural* 38:406-410. ISSN 0103-8478

Paesen, G.C. Adams,m P.L. , Harlos, K. Nuttall, P.A., Stuart, D.I. 1999. Tick histamine-binding proteins: isolation, cloning and three-dimensional structure. *Mollecular Cell* 3:661-671. ISSN 1097-2765.

Penaliggon, J. 1997. Getting to grips with fleas on pet dogs and cats. *Pesticide Outlook* 8:19-23. ISSN (electronic): 1465-8933.

Pereira, C.P.M, Oliveira P.R., Furquim, K.C.S., Bechara G.H, Camargo-Mathias M.I. (2011) Fipronil-induced cell death in salivary glands of *Rhipicephalus sanguineus* (Latreille, 1806) (Acari: Ixodidae) semi-engorged female. *Experimental Parasitology*,(October, 2010) 127:481-489 ISSN: 0014-4894

Pereira, C.P.M, Oliveira P.R., Furquim, K.C.S., Bechara G.H, Camargo-Mathias M.I. (2009) Effects of fipronil (active ingredient of Frontline) on salivary glands of *Rhipicephalus sanguineus* females (Latreille, 1806) (Acari: Ixodidae) semi-engorged female. *Veterinary Parasitology* (November, 2009) 166:124-130 . ISSN: 0304-4017

Roma G.C., Oliveira, P.R., Pizano, M.A., Camargo-Mathias M.I. (2009) Determination of LC50 of permethrin acaricide in semi-engorged females of the tick *Rhipicephalus sanguineus* (Acari: Ixodidae). E*xperimental Parasitology* (August, 2009) DOI 10.1016/j.exppara. 2009.08.001. ISSN: 0014-4894

Roma G.C., Furquim K.C.S., Bechara G.H., Camargo-Mathias M.I. (2010) Permethrin-induced morphological changes in oocytes of *Rhipicephalus sanguineus* (Acari: Ixodidae) semi-engorged females. *Food and Chemical Toxicology* (May, 2010) 48:825-830. ISSN: 0278-6915

Saito, K. C., Bechara, G.H., Nunes, E.T., Camargo-Mathias, M.I. 2005. Morphological, histological and ultrastructural studies of the ovary of the cattle tick *Boophilus microplus* (Canestrini, 1887) (Acari:Ixodidae). *Veterinary Parasitology* (September, 2004) 129:299-311. ISSN: 0304-4017

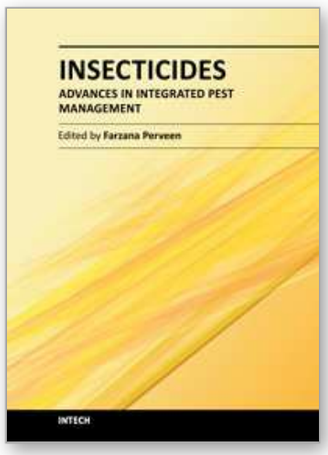
Sonenshine, D.E. 1991 The female reproductive system. In: Sonenshine, D.E. (Ed.) Biology of ticks. Oxford University Press. New York, 280-304.

Taylor, M.A. 2001. Recent developments in ectoparasiticides. Veterinary Journal, 161:253-268.

Tellam, R.L., Smith, D., Kemp, D.H., 1992. Vaccination against ticks. In:Young, W.K. (Ed.), Animal Parasite /control Using Biotechnology. CRC Press, Boca Raton, pp. 303-331.

Walker, A., Gletcher, J.d., Gill, H.S. 1985. Structural and histochemical changes in the salivary glands of *Rhipicephalus appendiculatus* during feeding. *International Journal for Parasitology* 15(1), 81-100.

Willadsen, P. 1997. Novel vaccines for ectoparasites. *Veterinary Parasitology* 71:209-222. ISSN: 0304-4017



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This book contains 30 Chapters divided into 5 Sections. Section A covers integrated pest management, alternative insect control strategies, ecological impact of insecticides as well as pesticides and drugs of forensic interest. Section B is dedicated to chemical control and health risks, applications for insecticides, metabolism of pesticides by human cytochrome p450, etc. Section C provides biochemical analyses of action of chlorfluazuron, pest control effects on seed yield, chemical ecology, quality control, development of ideal insecticide, insecticide resistance, etc. Section D reviews current analytical methods, electroanalysis of insecticides, insecticide activity and secondary metabolites. Section E provides data contributing to better understanding of biological control through Bacillus sphaericus and B. thuringiensis, entomopathogenic nematodes insecticides, vector-borne disease, etc. The subject matter in this book should attract the reader's concern to support rational decisions regarding the use of pesticides.

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