UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
INSTITUTO DE BIOCIÊNCIAS - RIO CLARO

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS
BIOLOGIA CELULAR E MOLECULAR

DINÂMICA DA VITELOGÊNESE DE CARRAPATOS *Amblyomma triste* (Koch, 1844) (ACARI: IXODIDAE).

PATRÍCIA ROSA DE OLIVEIRA

Dissertação apresentada ao Instituto de Biociências do Campus de Rio Claro, Universidade Estadual Paulista, como parte dos requisitos para obtenção do título de Mestre em Ciências Biológicas (Biologia Celular e Molecular).

Rio Claro/SP.
Janeiro – 2006.

UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
INSTITUTO DE BIOCIÊNCIAS - RIO CLARO

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS
BIOLOGIA CELULAR E MOLECULAR

DINÂMICA DA VITELOGÊNESE DE CARRAPATOS *Amblyomma triste* (Koch, 1844) (ACARI: IXODIDAE).

PATRÍCIA ROSA DE OLIVEIRA

Orientadora: Profa. Dra. MARIA IZABEL CAMARGO MATHIAS

Dissertação apresentada ao Instituto de Biociências do Campus de Rio Claro, Universidade Estadual Paulista, como parte dos requisitos para obtenção do título de Mestre em Ciências Biológicas (Biologia Celular e Molecular).

Rio Claro/SP.
Janeiro – 2006.

À minha mãe (mamy's), meu irmão (Glaubinho), meu pai e à minha grande Paixão (Paulo). DEDICO TODO O MEU TRABALHO!!!!

Viver é pensar... "Hoje levantei cedo pensando no que tenho a fazer antes que o relógio marque meia noite. É minha função escolher que tipo de dia vou ter hoje.... Se as coisas não saírem como planejei posso ficar feliz por ter outro dia para recomeçar. O dia está à minha frente esperando para ser o que eu quiser. E aqui estou eu, o escultor que pode dar forma. Tudo depende só de mim. "Sorria" mas não se esconda atrás deste sorriso. Mostre aquilo que você é. Sem medo. Existem pessoas que sonham. Viva. Tente. Felicidade é o resultado dessa tentativa. Ame acima de tudo. Ame a tudo e a todos. Deles depende a felicidade completa. Procure o que há de bom em tudo e em todos. Não faça dos defeitos uma distância e sim, uma aproximação. Aceite. A vida, as pessoas... Faça delas à sua razão de viver. Entenda os que pensam diferentemente de você. Não os reprove... Não corra... Para que tanta pressa? Corra apenas para dentro de você. Sonhe, mas não transforme esse sonho em fuga. Acredite! Espere! Sempre deve haver uma esperança. Sempre brilhará uma estrela. Chore! Lute! Faça aquilo que você gosta. Sinta o que há dentro de você. Ouça... Escute o que as pessoas têm a lhe dizer. É importante. Faça dos obstáculos degraus para aquilo que você acha supremo... Mas não esqueça daqueles que não conseguiram subir a escada da vida. Descubra aquilo de bom dentro de você. Procure acima de tudo ser gente. Eu também vou tentar. Sou feliz... Porque VOCÊ existe!" (CHARLES CHAPLIN).

“Não há limites para o homem que possui a capacidade de sonhar e a determinação, em transformar em realidade o sonho” (Autor desconhecido).

“Aja conforme diz. Diga conforme sente. Não jogue falso com sua consciência. Não a sufoque, forçando a engajar-se em ações por ela mesma desaprovada” (Sathya Sai Baba).

Agradecimentos

À Deus, pela oportunidade de finalizar mais uma etapa em meu caminho. Pelas inúmeras vezes que segurou em minha mão, me levantou, guiou e fortaleceu. Por suas bênçãos, pelo abrigo, proteção e pela luz em todos os momentos.

À minha mãe, que sempre está ao meu lado, que torce por mim como ninguém, que me ampara e que me ensina a lutar muito por todos os meus sonhos. Meu eterno obrigado!!!

Ao meu irmão e grande amigo, Glauber, companheiro nas diversões, na profissão e nas dificuldades, que luta para encontrar o seu caminho e que conquista cada vez mais o seu espaço. Nosso companheirismo me faz muito feliz! Obrigada por existir!

À minha grande Paixão, por ser o homem que me completa, que me ensina a amar, que me ama da maneira mais intensa e perfeita que pode existir, que sabe o que fazer para me agradar, que me conquista diariamente, que me permite sonhar, que me dá colo nos momentos difíceis, que me protege e cuida sempre que preciso e que se tornou o "homem da minha vida".

À meu pai. É impressionante perceber como o tempo e a distância contribuíram com a nossa relação. Respeito mútuo e confiança são alguns dos responsáveis por toda essa mudança! Você não conseguiria imaginar o quanto isso me faz feliz!!!

À minha orientadora e amiga, Bel, a quem devo meu progresso científico e aprendizado de como se deve fazer ciência, de quem vou guardar os preciosos ensinamentos pelo resto da vida. Você conquistou toda minha estima, respeito e admiração. Agradeço de forma muito especial por me orientar e lutar comigo para conseguirmos realizar nossos objetivos.

Ao Prof. Dr. Gervásio Henrique Bechara, pelo envio dos espécimens para mais um trabalho, mas principalmente por seu carinho e por sua gentileza, atenção e paciência sempre presentes em todas as mensagens.

A minha grande amiga Li, pela confiança, dedicação e atenção, pelo companheirismo e carinho, por dividir comigo suas alegrias e tristezas e por saber dar o verdadeiro significado à palavra amizade!!!

Ao meu amigo Emerson Arruda (Mê), que foi tão acolhedor nos momentos difíceis e que está sempre presente com todo o seu sorriso!!!!

Aos meus amigos e irmãos da Curva de Rio, Acerola, Capão, Frango e Rambo, obrigada pela acolhida, pelo companheirismo e por transformarem todo e qualquer momento em um grande acontecimento.

Aos amigos que compartilharam comigo tantos momentos... com tanto carinho, prestatividade e disposição: Rafael (que me ensinou que grandes diferenças não impedem grandes amizades), Thaísa (minha companheira mais que fiel de academia), Douglas (por sua presença mesmo distante), Ivan (pelas rizadas), Nena e Glei (pela ternura), Jordan (por seu carinho e atenção), Sunao (por sua amizade mais que atenciosa e pela poesia que eu "roubei" de você para essa dissertação), Flavito, Sorô, Gêmeas e Naty (pela bagunça e auto-astral de sempre).

Aos meus irmãozinhos da Tomahawk, Cupim, Tiuzinho, Ratinho e Naninha pela convivência harmoniosa e pela saudade deixada por aquele lar-doce-lar .

À minha sogra Cleuza Coghi e minha tia Fany Zuquim por terem me recebido tão bem nessa "nova família".

Aos meus cachorrinhos (Fred, Capitu e Iara), companheiros de todas as horas, que sempre me receberam com todo o seu carinho.

Às minhas amigas (Beletes): Gislaine (a mais que responsável!), Gaby (por todo seu carinho, sorriso,...), Amanda (por ter estado comigo naqueles momentos difícies de publicação) e à Deby, Karen, Sandra, Erika, Juliana e Lorena (pelas conversas, fofocas, rizadas...).

À Profa. Dra. Doralice, que me surpreendeu com a dedicação e amor a profissão.

À Profa. Dra. Carmem Sílvia Fontanetti e ao Prof. José Chaud Neto pela dedicação e exemplo de como ser docente.

À desenhista do Departamento de Biologia, Cris, pelas nossas conversas, pela sua ajuda, pela sua disposição e acima de tudo pela sua amizade.

Aos funcionários da Biblioteca, sempre atenciosos e educados, atendendo-nos da melhor forma possível.

A todos os funcionários do Departamento de Biologia que sempre me trataram tão bem... Lucila, Gerson, Rogilene, Mônika e Antônio.

Às pessoas fantásticas que passaram em meu caminho nestes anos, que deixaram muitas coisas e àquelas que passaram rápido, mas que não deixaram de ser importantes. Meu muito obrigado!

ÍNDICE

Pág.

RESUMO..1
ABSTRACT..3
INTRODUÇÃO GERAL...5
OBJETIVOS...13
MATERIAL E MÉTODOS..15
1. Microscopia Eletrônica de Varredura (MEV)..15
2. Inclusão em resina (Historesina)...16
3. Histoquímica..17
3.1. Técnica do Azul de Bromofenol...17
3.2. Técnica do Azul de Nilo...17
3.3. Técnica do PAS 17
4. Microscopia Eletrônica de Transmissão (MET)......................................18
5. Citoquímica Ultra-estrutural...19
5.1. Carboidratos - Método de Thiery 19
5.2. Proteínas - Técnica da Prata Amoniacal 20
5.3. Lipídios - Técnica do Ósmio-Imidazol 20
RESULTADOS 22
Capítulo 1: *Amblyomma triste* (Koch, 1844) (Acari: Ixodidae): Morphological description of the ovary and of vitellogenesis…………………………………………………………….24
1. Introduction…………………………………………………………….26
2. Material and Methods…………………………………………….26
2.1. Scanning Electron Microscopy (SEM)…………………….....27
2.2. Histology...…………………………………………………..27
2.3 Histochemistry…………………………………………........27
2.4 Transmission Electron Microscopy (TEM)………………..27
3. Results………………………………………………………………..28
3.1. Ultramorphology……………………………………………...28
3.2. Histology……………………………………………………..28
3.3. Oocyte classification……………………………………......28

3.4. Histochemistry…………………………………………...………29
4. Discussion……………………………………………………….....…30
5. Acknowledgments……………………………………………………34
6. Legend of Figures ……..……………………………………………..34
7. Table…….……………………………………………………………39
8. References.………….………………………………………………40
Capítulo 2: Female ticks of *Amblyomma triste* (Koch, 1844) (Acari: Ixodidae). Ovaries ultrastructural analysis…………………………..……...43
1. Introduction……………………………………………………..…..…46
2. Material and Methods………………………………………………..46
2. 1. Transmission Electron Microscopy (TEM)……………...47
3. Results…..………………………………………………………..…....47
4. Discussion…………………………………………………………..49
5. Acknowledgments…………………………………………………....53
6. Legend of Figures…………………………………..……………..53
7. References……………………………………………..………...59
Capítulo 3: Vitellogenesis in the tick *Amblyomma triste* (Koch, 1844) (Acari: Ixodidae). Determining a new role for pedicel cells......................62
1. Introduction……………………………………………………………64
2. Material and Methods………………………………………………...64
2.1. Transmission Electron Microscopy (TEM)………..……...65
3. Results………………………………………………………......…65
4. Discussion……………………………………………………………..67
5. Acknowledgments………………………………………………..…..…70
6. Legend of Figures ……………………………………………….....71
7. References…………………………………………………………..…74
Capítulo 4: Ultrastructural detection of proteins, lipids and carbohydrates in oocytes of *Amblyomma triste* (Kock, 1844) (Acari; Ixodidae) during the vitellogenesis process………………………………..76
1. Introduction……………………………………………………..…..78
2. Material and Methods….…………………………………………...…79

2.1. Ultrastructural cytochemistry for the detection of carbohydrates……………………………………………………..…..79

2.2. Ultrastructural cytochemistry for the detection of lipids…………………………………………………………....80

2.3. Ultrastructural cytochemistry for the detection of basic proteins…………………………………………………….......80

3. Results………………………………………………………….....81

4. Discussion………………………………………………………...…87

5. Acknowledgments…..…………………………………………........95

6. Legend of Figures...95

7. References…….………………………………..…………….....103

DISCUSSÃO GERAL..106

CONCLUSÕES..114

REFERÊNCIAS BIBLIOGRÁFICAS…………………………………………..…116

RESUMO

O presente estudo apresenta a análise morfológica, histológica e ultra-estrutural do ovário bem como a dinâmica da vitelogênese de fêmeas de carrapatos *Amblyomma triste*. O ovário dessa espécie é do tipo panoístico, não possuindo, portanto, células nutridoras nem foliculares. O ovário é composto por ovócitos que estão presos à parede do ovário, camada única de células epiteliais, através do pedicelo, estrutura celular com função de sintetizar e fornecer substâncias para o desenvolvimento dos mesmos. Os ovócitos foram identificados e classificados em quatro estágios diferentes de desenvolvimento. A classificação foi realizada baseada na presença de quatro características morfológicas: aspecto citoplasmático; localização da vesícula germinal; presença, quantidade e constituição dos grânulos de vitelo e presença de cório. Nos ovócitos de *A. triste* ocorre a síntese endógena e principalmente exógena dos elementos do vitelo. Em carrapatos *A. triste,* os lipídios dos ovócitos têm origem: a) exógena, em todos os estágios, uma vez que os elementos são captados da hemolinfa via superfície do ovócito e/ou provenientes das células do pedicelo e, b) endógena, que ocorre somente nos ovócitos III e IV. As

proteínas do vitelo dos ovócitos de *A. triste* têm origem: a) exógena, nos ovócitos II, III e IV, via células do pedicelo que atuariam como fornecedoras deste elemento e também por meio da captação da hemolinfa pelo próprio ovócito e, b) endógena, nos ovócitos IV. Já os polissacarídios teriam origem a partir de duas fontes exógenas, a primeira com a produção e/ou transporte desse elemento via células do pedicelo, e a segunda, por meio da captação da hemolinfa via membrana do ovócito. No presente trabalho, a função do pedicelo como fornecedor de elementos para os ovócitos durante a vitelogênese fica estabelecida nessa espécie de carrapatos.

ABSTRACT

The present study presents the morphological, histological and ultrastructural analysis as well as the dynamics of vitellogenesis in females of the tick *Amblyomma triste*. The ovary of this species is of the panoistic type, not presenting nurse nor follicular cells. The ovary is composed of oocytes which remain attached to wall of the ovary (single layer of epithelial cells) through the pedicel, a cellular structure responsible for synthesizing and providing substances to oocytes, promoting their development. The oocytes had been identified and classified into four different developmental stages. The classification was carried out based on the presence of four morphologic characteristics: cytoplasm appearance; site of the germ vesicle; presence, quantity and constitution of the yolk granules and presence of chorium. In the oocytes of *A. triste* occur the endogenous synthesis and mainly exogenous incorporation of yolk elements. The lipids have two different origin: a) exogenous, in all the oocytes, captured from the hemolymph through the oocyte surface and produced by pedicel cells and b) endogenous, only stages III and IV. The proteins have origin: a) exogenous, in the oocytes II, III e IV, where the pedicel cells contribute with the protein supply for the yolk constitution and also

through the capture of this element from the hemolymph by the oocyte and, b) endogenous, in the oocytes IV. The polysaccharides would have two exogenous sources: the production and transportation of this element through the pedicel cells; and the capture of the hemolymph through the oocyte plasmic membrane. The present study determines the role of the pedicel as a provider of yolk elements to these tick species oocytes.

INTRODUÇÃO GERAL

Os Acari, subclasse dos aracnídeos onde se encontram os ácaros e os carrapatos (BARNES; RUPPERT, 1996), constituem um grupo formado por indivíduos que variam de pequenos a microscópicos, apresentando cabeça, tórax e abdômen fundidos e não segmentados (STORER; USINGER, 1977; BARNES; RUPPERT, 1996). Segundo Sonenshine (1991), apesar de não terem nenhuma adaptação para o vôo, estes indivíduos concorrem com os insetos em diversidade de espécies e adaptações.

Carrapatos são classificados em duas famílias maiores: a Ixodidae e a Argasidae. A primeira caracteriza-se pela presença de peças bucais inseridas em uma depressão na borda anterior do corpo dos indivíduos, o que permite que os mesmos sejam consumidores vorazes de sangue, bem como pela presença de um escudo quitinoso, que nos machos recobre quase toda a superfície dorsal e, nas fêmeas, não vai além da metade ou de um terço da área dorsal dos indivíduos não alimentados. Já a segunda, caracteriza-se pela ausência de escudo e pela presença das peças bucais localizadas, nos adultos e nas ninfas, na face inferior do corpo, o que explica o fato desses carrapatos consumirem relativamente pouco sangue em contraste com os indivíduos da outra família citada (WALKER,1994; WALL; SHEARER, 1997). Há ainda uma

terceira e menor família, Nutalliellidae, constituída apenas por uma espécie (SAUER et al*.,* 1999).

Os carrapatos adultos apresentam pernas com seis segmentos, em cuja extremidade há um pedúnculo, que pode ser curto ou longo e no qual se inserem duas garras bem desenvolvidas. Os ixodídeos possuem na base das garras uma expansão em forma de disco denominada pulvilo (REY, 2001)

Os carrapatos são considerados parasitas de animais vertebrados e podem ter vários hospedeiros dos quais irão se alimentar. A localização dos hospedeiros é facilitada pela elevada eficiência de seu sistema sensorial que detecta odor, vibração, mudanças de temperatura entre outros (SONENSHINE, 1991).

Durante seu ciclo biológico, os carrapatos podem passar longos períodos fora de seus hospedeiros, abrigados entre a vegetação e fendas no solo (WALKER,1994). O fator principal que explica a habilidade dos carrapatos em sobreviver por longos períodos sem alimentação, é a capacidade de manter a quantidade de água em seu corpo e de absorvê-la do ar insaturado do ambiente (RUDOLPH; KNULLE, 1974; MCMULLEN et al., 1976; NEEDHAM et al., 1990; NEEDHAM; TEEL, 1991; SIGAL et al., 1999).

De acordo com os estudos realizados por Harwood e James (1979), os carrapatos de maneira geral possuem extraordinária capacidade de atuar como vetores de vírus, bactérias, ricketsias e protozoários, provocando doenças nos seus hospedeiros, os quais podem ser: animais domésticos, silvestres, aves e até mesmo o homem, por apresentarem as seguintes características biológicas:

- hematofagismo em todas as fases do desenvolvimento, o que aumenta a sua eficiência como vetor;
- fixação profunda nos hospedeiros, propiciando dificuldades de remoção e facilitando a dispersão por aves e mamíferos;
- ingurgitamento lento, propiciando tempo para adquirir e inocular patógenos;
- adaptação a diferentes hospedeiros, possibilitando veiculação de patógenos entre diferentes espécies;

- longevidade dos estágios em seus ambientes, propiciando tempo para multiplicação dos patógenos;
- transmissão transovariana de microrganismos, permitindo a geração sucessiva de indivíduos com potencial de transmitir os microrganismos e funcionar como eficientes reservatórios;
- poucos inimigos naturais, pela eficiente adaptação ao ambiente tropical;
- grande esclerotização da cutícula, propiciando resistência a adversidade climática;
- grande potencial biótico, possibilitando a perpetuação da espécie.

O corpo dos carrapatos não é completamente esclerotizado sendo capaz de uma grande expansão quando ingurgitado com sangue, podendo ainda ter pêlos ou cerdas (BARNES; RUPPERT, 1996).

A fixação do carrapato ocorre quando o seu hipostômio penetra na pele do hospedeiro, como conseqüência da ação combinada entre as quelíceras e a saliva. A digestão dos tecidos ao redor do canal de penetração do hipostômio causa ruptura dos capilares e vasos linfáticos do mesmo. Os carrapatos se alimentam por sucção do sangue do hospedeiro, alternada com a eliminação da saliva produzida pelas suas glândulas salivares. Grande volume de saliva é eliminado no final do processo de ingurgitamento (BALASHOV, 1972) causando o aumento da permeabilidade vascular do hospedeiro e, conseqüentemente aumentando o fluxo de sangue para o carrapato (RIBEIRO, 1987).

Os carrapatos argasídeos alimentam-se rapidamente de seus hospedeiros, aumentando de tamanho em minutos ou em poucas horas. Já os ixodídeos alimentam-se por longo tempo, variando de poucos dias a diversas semanas. Os ixodídeos concentram o sangue que consomem de seu hospedeiro, portanto conseguem eliminar o excesso de água deste sangue, ou por transpiração corporal, ou pelos restos fecais, ou ainda através de suas glândulas salivares. A voracidade pela qual os carrapatos consomem o sangue pode resultar em sérios danos para o seu hospedeiro, principalmente quando há muitos carrapatos infestando um só indivíduo (SONENSHINE, 1991).

Moorhouse, Tatchell (1966) e Balashov (1972) descreveram as conseqüências das picadas de carrapatos: espoliação direta pelo hematofagismo; indireta pela compressão de células e tecidos; ação mecânica pela dilaceração de células e tecidos; ação tóxica pela saliva, além da depreciação do couro. Ross (1926), Zumpt, Glajchen (1950), Wall e Shearer (1997) ainda destacaram que pode ocorrer paralisia no hospedeiro e que a ferida formada permitiria a entrada de microrganismos, os quais poderiam causar infecções secundárias como miíases e abcessos. A paralisia causada tem caráter motor, progressivo e é atribuída a uma toxina que pode ser produzida pelo ovário dos carrapatos (WALL; SHEARER, 1997).

Embora a extensão da lesão no hospedeiro e a capacidade de ingestão de sangue pelo carrapato variem consideravelmente; independentemente da fase de desenvolvimento do indivíduo, as peças bucais das larvas, das ninfas e dos adultos de determinadas espécies penetram na pele do hospedeiro na mesma profundidade (TATCHELL; MOORHOUSE, 1970).

Dentro da família Ixodidae encontra-se o gênero *Amblyomma*, que compreende indivíduos com capítulo longo e com o segundo segmento do palpo pelo menos duas vezes mais longo do que largo. Espécies deste gênero parasitam uma grande variedade de hospedeiros, tais como: porcos, galinhas, roedores, coelhos, capivaras, cervos, cães e mesmo anfíbios, répteis e o homem (SEMTNER; HAIR*,* 1973; ANDERSON; MAGNARELLI 1980). Distribuem-se por todo mundo, principalmente em regiões tropicais e subtropicais. Cerca de 30 espécies são encontradas no Brasil (REY, 2001), entre elas, o *Amblyomma triste* (Kock, 1844).

O *A. triste* (Kock, 1844) ocupou originalmente toda a Costa do Atlântico, a partir do Golfo do México, chegando a atingir até 150 milhas da costa (BISHOP; HIXSON, 1936) e assim, em 1970, tornou-se praga de significado econômico (SEMTNER; HAIR*,* 1973).

O carrapato *A. triste* é caracterizado por marcante antropofilia que é reconhecida tanto no Brasil (São Paulo, Mato Grosso e Mato Grosso do Sul) quanto em outros países da América do Sul (Argentina, Uruguai, Equador e Venezuela) (SEMTNER; HAIR*,* 1973; ANDERSON; MAGNARELLI, 1980).

Os primeiros animais infestados por esta espécie de carrapato foram as antas (*Tapirus* sp) e, posteriormente, cachorros, gambás (*Didelphis marsupialis*) e capivaras (*Hydrochaeris hydrochaeris*) (SEMTNER; HAIR,1973).

Estudos realizados nas regiões alagadas pela represa da Estação Hidrelétrica de Porto Primavera, fronteira do Estado de São Paulo com o Mato Grosso do Sul, registraram espécimens do cervo do Pantanal (*Blastocerus dichotomus*) como hospedeiro de adultos de *A. triste* (CONTI DÍAZ, 2001; SZABO et al., 2003).

No entanto, no Uruguai, onde não existem cervo do Pantanal, os espécimens adultos de *A. triste* foram encontrados parasitando cães e o homem (CONTI DÍAZ, 2001; VENZAL et. al., 2003), enquanto que os estágios imaturos parasitavam marsupiais (*Monodelphis dimidiata*) e roedores (*Scapteromys tumidus, Oxymycterus nasutus* e *Oligorysomys flavescens*) (VENZAL; FREGUEIRO, 1999).

O *A. triste* é o principal ou mesmo, o único vetor da patologia riquetsiose causada por *Rickettsia conorii.* Em 1990, foram registrados os primeiros três casos de riquetsiose causada por esse agente etiológico (CONTI DÍAZ, 1990, 2001). Nessa riquetsiose, os pacientes apresentam um quadro agudo caracterizado pela presença de uma lesão ganglionar cutânea com um centro de cor negra (a clássica “mancha negra”) no local da picada do carrapato. Pouco tempo depois, é diagnosticada no paciente febre alta acompanhada freqüentemente de mal estar generalizado e intensa dor-de-cabeça (SEMTNER; HAIR,1973).

Segundo Freitas (1982), este ixodídeo necessita de três hospedeiros para completar o seu ciclo biológico. As fêmeas ingurgitadas destacam-se do hospedeiro e procuram abrigo para ovipositarem cerca de 2000 a 3000 ovos. O número de ovos produzidos depende do volume de sangue ingerido e do tamanho dos próprios ovos. Em geral, fêmeas que consomem grande quantidade de sangue produzem mais ovos (SONENSHINE, 1991).

Os ovos atingem a maturidade após 20 a 60 dias, de onde emergem as larvas com seis pernas; estas prendem-se ao primeiro hospedeiro do ciclo. Uma vez repletas de sangue elas abandonam esse hospedeiro, mudam o

exoesqueleto e passam para o estágio de ninfa com oito pernas. As ninfas logo se prendem e se alimentam no segundo hospedeiro, transformando-se em adultos que procuram o terceiro hospedeiro para se fixar e se alimentar até a oviposição. O ciclo vital pode completar-se em dois meses (FREITAS, 1982; WALKER,1994).

Métodos eficazes para controlar carrapatos, que transmitem ou não doenças no ato de sua alimentação, estão sendo investigados e incluem vacinas, sprays carrapaticidas e uso tópico (PETER; BROSSARD, 1998; BECHARA, 2003), o uso de predadores (aves) e parasitas (fungos ou bactérias) (LIPA, 1971), o uso de feromônios (DEBRUYNE; GUERIN, 1994) e no caso do combate aos carrapatos presentes em bovinos, utiliza-se a rotação de pastagens (LIPA, 1971). Também estão sendo utilizados o controle climático, através de temperaturas abaixo de 17,5^{O}C, precipitações pluviométricas anuais abaixo de 400mm e umidade relativa abaixo de 70% (LIPA, 1971), e o cruzamento genético de diferentes raças de gado visando aumentar naturalmente a resistência do animal (PETER; BROSSARD, 1998).

Na tentativa de minimizar a ação dos carrapatos, tem-se feito também uso do controle químico. Vários compostos já tiveram sua ação comprovada (NEWTON, 1967; DAVEY et al., 1998), porém alguns deles acarretaram no desenvolvimento de resistência pelos carrapatos. É importante dizer que, uma vez que tenha sido comprovada essa resistência a um determinado acaricida, ela é irreversível e o emprego dessa mesma base química não terá qualquer efeito para o controle futuro (GRILLO,1976).

Estudos mais avançados têm aberto a perspectiva para o controle imunológico, através da identificação, isolamento e síntese de proteínas protetoras imunogênicas de tecidos e órgãos de carrapatos, principalmente dos aparelhos digestório e reprodutor, e seu emprego em vacinas (TELLAM et al.,1992; WILLADSEN, 1997). Para tanto, torna-se fundamental conhecer seus aspectos morfofisiológicos.

Muitas investigações vêm sendo realizadas com carrapatos, principalmente enfocando aspectos bioeconômicos, ecologia além de estudos com anatomia externa, com vistas à taxonomia (HOOGSTRAAL,

1956; TILL, 1961; OLIVEIRA et al., 2004). No que diz respeito à anatomia interna, os estudos são mais escassos e a bibliografia muito antiga (ROBINSON; DAVIDSON, 1913; DOUGLAS, 1943; VITZTHUM, 1943). Recentemente, alguns autores têm se dedicado a realizar o estudo morfológico do sistema reprodutor e das glândulas salivares de várias espécies de carrapatos de importância médico-veterinária (DENARDI et al., 2004; OLIVEIRA et al., 2005; SAITO et al., 2005; NUNES et al., 2005; OLIVEIRA et al., in press).

Nos carrapatos, o sistema reprodutor feminino consiste de um ovário tubular único e localizado na região posterior do corpo, um par de ovidutos, um útero, a vagina e um par de glândulas tubulares acessórias desembocando numa abertura genital. Na região posterior e muscular encontra-se a vagina cervical e na região anterior, a vagina vestibular que é revestida internamente por uma cutícula pregueada. Há também um receptáculo seminal que se conecta a vagina cervical e que armazena os espermatozóides introduzidos durante a cópula.

Os ixodídeos apresentam o ovário disposto em forma de ferradura e um segundo par de glândulas acessórias, as glândulas acessórias lobulares, localizadas ao redor da vagina cervical (SONENSHINE, 1991).

Nos argasídeos, o ovário é menor do que nos ixodídeos, ocupando apenas uma secção transversal da região posterior do corpo. Os ovidutos são tão longos quanto nos ixodídeos, porém, nos argasídeos aumenta muito de tamanho próximo de sua junção com o útero. Um par de glândulas tubulares acessórias é encontrado próximo à região de contato vagina cervical/vagina vestibular, no entanto os carrapatos argasídeos não possuem glândulas lobulares acessórias (SONENSHINE, 1991).

O ovário dos carrapatos em geral possui algumas diferenças morfológicas e histológicas quando consideradas as famílias Argasidae e Ixodidae e até mesmo dentro dos ixodídeos (TILL,1961; DENARDI et al., 2004; OLIVEIRA et al., 2005; SAITO et al., 2005; OLIVEIRA et al., in press).

O ovário dos carrapatos é do tipo panoístico, como descrito em insetos, não possuindo, portanto, células nutridoras (DENARDI et al., 2004; OLIVEIRA

et al., 2005; SAITO et al*.,* 2005; OLIVEIRA et al., in press). Este é descrito como sendo constituído por uma parede de células epiteliais, pelos pedicelos, estruturas celulares que prendem os ovócitos à sua parede e por um grande número de ovócitos, os quais passam por vários estágios de desenvolvimento até estarem prontos para a oviposição (TILL, 1961; SAID, 1992; DENARDI et al., 2004; OLIVEIRA et al., 2005; SAITO et al*.,* 2005; OLIVEIRA et al., in press).

Em *A. cajennense*, foi descrita a ocorrência de ovócitos em cinco diferentes estágios de desenvolvimento (DENARDI et al., 2004). Nos ovócitos de *Rhipicephalus sanguineus* foram encontrados cinco estágios de desenvolvimento além de alguns ovócitos em processo de degeneração (OLIVEIRA et al., 2005) e em *Boophilus microplus,* de seis estágios de desenvolvimento (SAITO et al., 2005).

OBJETIVOS

Diante das informações acima expostas e considerando as diferenças encontradas nos ovários de outras espécies de carrapatos, a necessidade de se estabelecer a função do pedicelo e a ausência de estudos sobre a dinâmica da vitelogênese em fêmeas de carrapatos *Amblyomma triste,* o presente estudo teve como objetivos principais:

a) Caracterizar a ultramorfologia e a histologia do ovário por meio da aplicação de técnicas de Microscopia Eletrônica de Varredura (MEV) e de inclusão em resina;

b) Detectar os elementos constituintes do vitelo dos ovócitos desta espécie, empregando-se os testes histoquímicos de Azul de Nilo, Azul de Bromofenol e PAS,

c) Investigar as reais funções do pedicelo nos carrapatos por meio do estudo ultra-estrutural do ovário empregando-se técnicas de Microscopia Eletrônica de Transmissão (MET);

d) Detectar a origem dos diversos elementos que compõem o vitelo dos ovócitos desta espécie de carrapatos por meio de estudo citoquímico do ovário aplicando-se o método de Thiery, e as técnicas da Prata Amoniacal e do Ósmio-Imidazol.

MATERIAL E MÉTODOS

No presente estudo foram utilizadas fêmeas semi-ingurgitadas de carrapatos, da espécie *Amblyomma triste.* Os espécimens foram fornecidos pelo Prof. Dr. Gervásio Henrique Bechara obtidos a partir de colônia mantida em laboratório, em condições controladas (29º C, 80% de umidade e fotoperíodo de 12 h) em estufa BOD no Departamento de Patologia Veterinária da UNESP - Campus de Jaboticabal, S.P.

Para o desenvolvimento do trabalho foram utilizados os equipamentos disponíveis nas dependências dos laboratórios de Histologia e de Microscopia Eletrônica, do Departamento de Biologia, da UNESP – Campus de Rio Claro, SP.

1. Microscopia Eletrônica de Varredura (MEV)

Os indivíduos foram anestesiados a frio (choque térmico) e colocados em placa de Petri contendo solução fisiológica tamponada com fosfato-PBS (NaCl 7.5 g/L, Na_2HPO_4 2,38 g/L e KH_2PO_4 2,72 g/L). Sob lupa, os ovários foram retirados com auxílio de pinças e micro-tesouras e, então, foram

colocados em solução fixadora de Karnovsky por 2 horas e, a seguir, passaram por série crescente de banhos com acetona 70%, 80%, 90%, 95%, 100% (duas vezes) com duração de 10 minutos cada. Em seguida, foram transferidos para solução de álcool 100% com acetona P.A. na proporção de 1:1 onde permaneceram durante 15 minutos. Depois, foram submetidos a um banho rápido com acetona P.A.

Na seqüência, o material foi levado ao ponto crítico e posteriormente, foi fixado a um suporte de metal com fita adesiva dupla face onde recebeu camadas alternadas de ouro e de carbono.

A seguir, o material foi analisado e fotografado em Microscópio Eletrônico de Varredura PHILLIPS 505, SEM.

2. Inclusão em resina (Historesina)

Os indivíduos foram anestesiados a frio (choque térmico) e colocados em placa de Petri contendo solução fisiológica tamponada com fosfato-PBS (NaCl 7.5 g/L, Na_2HPO_4 2,38 g/L e KH_2PO_4 2,72 g/L). Sob lupa, os ovários foram retirados com auxílio de pinças e micro-tesouras e, então, permaneceram, por um período de 24 horas em solução fixadora de paraformaldeído 4%. Logo após, o material foi transferido para solução tampão fosfato de sódio onde permaneceu por mais 24 horas.

Procedeu-se então a desidratação em série crescente de álcool etílico a 70%, 80%, 90% e 95% . Cada banho teve duração de 30 minutos. Em seguida, o material foi embebido em resina Leica por 24 horas e incluído em moldes plásticos contendo resina Leica mais polimerizador. Posteriormente, os blocos foram colocados em suportes de madeira para secção em micrótomo de onde foram obtidos cortes com a espessura de 5 µm que foram recolhidos em lâminas de vidro previamente limpas.

As lâminas contendo as secções foram coradas pela Hematoxilina durante 5 minutos. Depois de lavadas por 5 minutos em água corrente, foram coradas com Eosina aquosa durante 5 minutos e lavadas em água corrente novamente.

Após secagem ao ar livre, em suportes de madeira, as lâminas foram mergulhadas rapidamente em xilol e, em seguida, foram montadas com bálsamo do Canadá e recobertas com lamínula.

As lâminas obtidas foram examinadas e analisadas em microscópio de luz.

3. Histoquímica

As técnicas histoquímicas foram aplicadas para detectar a presença de proteínas, polissacarídeos e lipídios nos ovários de fêmeas de *Amblyomma triste*.

3.1. Técnica do Azul de Bromofenol para detecção de proteínas totais (Segundo PEARSE, 1985):

As lâminas contendo as secções foram coradas com Azul de Bromofenol por duas horas à temperatura ambiente. Após serem lavadas com ácido acético 0,5% por 5 minutos e com água corrente por 15 minutos, as mesmas passaram rapidamente por uma solução de álcool butílico terciário.

A seguir, foram secas ao ar livre, diafanizadas e montadas em bálsamo do Canadá.

3.2. Técnica do Azul de Nilo para detecção de lipídios (Segundo LISON, 1985):

O material foi fixado em Formol Cálcio por 24 horas.

As lâminas foram coradas por 5 minutos a 37°C com azul de Nilo. Após lavadas em água corrente, permaneceram durante 1 minuto em solução de ácido acético 1%. Em seguida, foi realizada a contracoloração rápida com hematoxilina em algumas lâminas, a fim de evidenciar os núcleos. Depois de secas, as lâminas foram montadas com glicerina e recobertas com lamínula.

3.3. Técnica do PAS para detecção de polissacarídeos (Segundo JUNQUEIRA; JUNQUEIRA, 1983):

As lâminas contendo as secções permaneceram 10 minutos em solução de ácido periódico 0,4%. Em seguida, foram lavadas rapidamente em água destilada e submetidas ao reagente de Schiff durante 1 hora, no escuro. Posteriormente, foram feitas três lavagens em água sulfurosa por 3 minutos cada e, a seguir, as lâminas foram lavadas em água corrente por 30 minutos.

Após secagem, as lâminas foram diafanizadas em xilol e montadas com Bálsamo do Canadá.

4. Microscopia Eletrônica de Transmissão (MET)

Os indivíduos foram dissecados em glutaraldeído 0,5%, onde tiveram os ovários retirados e fixados em glutaraldeído 2,5% à temperatura ambiente, por 2 horas. Em seguida, o material passou por duas lavagens de 15 minutos cada, em solução tampão de cacodilato de sódio à 0,1M. O material foi pós-fixado em tetróxido de ósmio 1% em solução tampão cacodilato de sódio 0,1M. Novamente foram realizadas duas lavagens em solução de cacodilato de sódio 0,1M por 15 minutos cada.

A seguir, o material passou por um banho de álcool 10% durante 15 minutos e, posteriormente, foi contrastado com acetato de uranila 2%. Posteriormente, foi realizada a desidratação em série crescente de acetonas 50%, 70%, 90%, 95% e 100% (esta última duas vezes), com duração de 10 minutos cada e acetona pura com duração de 15 minutos. Logo após, o material foi submetido a mistura de acetona e resina na proporção de 1:1 onde permaneceu por 12 horas.

Finalmente, o material foi incluído em resina pura contendo catalisador e colocado em estufa à 60°C por 24 horas.

O material foi seccionado em ultramicrótomo e as secções foram colocadas em grades de cobre. A seguir, as mesmas foram contrastadas com

acetato de uranila e citrato de chumbo durante 25 e 10 minutos respectivamente.

As grades contendo as secções foram examinadas e fotografadas em Microscópio Eletrônico de Transmissão PHILLIPS 100, TEM.

5. Citoquímica Ultra-estrutural

Testes citoquímicos foram aplicados para detectar em nível ultra-estrutural a presença de carboidratos (Thiery, segundo HADDAD et al., 1998); lipídios (Angermuller; De Fahimi, segundo HADDAD et al., 1998) e proteínas (Macrae; Meetz, segundo HADDAD et al., 1998).

5.1. Carboidratos - Método de Thiery (Segundo HADDAD et al., 1998):

Os ovários foram dissecados em placas de Petri contendo solução fisiológica tamponada com fosfato-PBS (NaCl 7,5 g/L, Na_2HPO_4 2,38 g/L e KH_2PO_4 2,72 g/L), e em glutaraldeído 2,5% em tampão cacodilato 0,1M. Posteriormente, foram desidratados em acetona e incluídos em resina Epon.

Os cortes ultra finos obtidos em ultramicrótomo, foram recolhidos em telas de ouro de 400 mesh. As telas contendo as secções flutuaram em gotas de solução de ácido periódico 1% durante 30 minutos. Em seguida, foram lavadas em água destilada, sendo duas lavagens rápidas e duas por 10 minutos cada com agitação. Posteriormente, as telas flutuaram em gotas de solução de tiosemicarbazida 1% em ácido acético 10% durante 22 horas para detecção de glicogênio, mucopolissacarídeos e glicoproteínas. A seguir, as telas foram lavadas em ácido periódico 10% por 3 vezes durante 10 minutos, em ácido acético 5% durante 5 minutos, em 2% durante 5 minutos e três vezes em água destilada durante 10 minutos cada.

Na seqüência, as telas foram colocadas para flutuar em solução de proteinato de prata 1% durante 30 minutos, no escuro e à temperatura ambiente. Posteriormente, foram lavadas em água destilada e, sem

contrastação prévia com acetato de uranila ou citrato de chumbo, foram observadas ao Microscópio Eletrônico de Transmissão PHILLIPS TEM 100

5.2. Proteínas - Técnica da Prata Amoniacal (MACRAE & MELTZ, 1970) (Segundo HADDAD et al., 1998):

Os indivíduos foram dissecados em glutaraldeído 0,5%, onde tiveram os ovários retirados e fixados em glutaraldeído 2,5% em tampão cacodilato 0,1M, “over night”. O material foi então lavado exaustivamente em água destilada.

Em seguida foi realizada a incubação em solução de prata amoniacal (adição gradual de nitrato de prata ($AgNO_3$) a 10% em solução concentrada de hidróxido de amônio (NH_4OH), até a observação de ligeira turvação). A incubação foi feita à temperatura ambiente por 5 minutos. Foram realizadas, então, três lavagens em água destilada, com posterior incubação em solução de formaldeído 3%, durante 5 minutos, onde o material adquiriu coloração marrom. Foram feitas mais três lavagens em água destilada e, em seguida, a pós-fixação em tetróxido de ósmio 1% em tampão cacodilato 0,1 M por 2 horas, no escuro à temperatura ambiente. Em seguida, o material foi lavado em tampão cacodilato 0,1M durante 10 minutos, desidratado em acetona e incluído em resina Epon.

As secções foram obtidas em ultramicrótomo e coletadas em telas de cobre e, sem contrastação, foram observadas ao Microscópio Eletrônico de Transmissão PHILLIPS TEM 100.

5.3. Lipídios - Técnica do Ósmio-Imidazol (Segundo HADDAD et al., 1998):

Os ovários depois de retirados foram fixados em glutaraldeído a 2,5% e em tampão cacodilato 0,1M, pH7,2, “over night”.

A seguir, o material foi lavado em tampão cacodilato 0,1M, pH 7,2, durante 10 minutos e em tampão imidazol 0,1M, pH 7,5, durante 10 minutos.

A pós-fixação foi feita em tetróxido de ósmio a 2% e em tampão imidazol 0,1M, pH 7,5, à temperatura ambiente e no escuro, durante 30 minutos. Em

seguida, foram realizadas duas lavagens em tampão imidazol 0,1M, pH 7,5 por 10 minutos cada. A desidratação ocorreu em acetona e a inclusão, em resina Epon.

Os cortes ultra finos, sem contrastação, foram observados ao Microscópio Eletrônico de Transmissão PHILLIPS TEM 100. As inclusões lipídicas apareceram eletrondensas, devido à ligação com o ósmio.

RESULTADOS

Os resultados estão apresentados sob a forma de capítulos onde cada um contém um artigo já publicado ou submetido a periódico internacional especializado na área. Cada artigo traz os resultados dos diferentes aspectos dos ovários de carrapatos *Amblyomma triste.*

Desta forma, a dissertação está composta por quatro capítulos, onde são apresentados os seguintes artigos:

Capítulo 1:

Título do artigo: *Amblyomma triste* (Koch, 1844) (Acari: Ixodidae): Morphological description of the ovary and of vitellogenesis.

Autores: Patrícia Rosa de Oliveira; Maria Izabel Camargo Mathias e Gervásio Henrique Bechara.

Periódico: Experimental Parasitology.

Situação: Submetido e aceito.

Capítulo 2:

Título do artigo: Female ticks of *Amblyomma triste* (Koch, 1844) (Acari: Ixodidae). Ovaries ultrastructural analysis.
Autores: Patrícia Rosa de Oliveira; Maria Izabel Camargo Mathias e Gervásio Henrique Bechara.
Periódico: Micron.
Situação: Submetido e aceito.

Capítulo 3:

Título do artigo: Vitellogenesis in the tick *Amblyomma triste* (Koch, 1844) (Acari: Ixodidae). Determining a new role for pedicel cells.
Autores: Patrícia Rosa de Oliveira; Maria Izabel Camargo Mathias e Gervásio Henrique Bechara.
Periódico: Veterinary Parasitology.
Situação: Submetido.

Capítulo 4:

Título do artigo: Ultrastructural detection of proteins, lipids and carbohydrates in oocytes of *Amblyomma triste* (Kock, 1844) (Acari; Ixodidae) during the vitellogenesis process.
Autores*:* Patrícia Rosa Oliveira; Amanda Juliana Ricardo; Maria Izabel Camargo Mathias e Gervásio Henrique Bechara.
Periódico: Veterinary Parasitology.
Situação: Submetido.

Capítulo 1:

Título do artigo: *Amblyomma triste* (Koch, 1844) (Acari: Ixodidae): Morphological description of the ovary and of vitellogenesis.

Autores: Patrícia Rosa de Oliveira; Maria Izabel Camargo Mathias e Gervásio Henrique Bechara.

Periódico: Experimental Parasitology.

Situação: Submetido e aceito.

Resumo: O presente estudo apresenta a morfologia, histologia e a dinâmica da vitelogênese em fêmeas de carrapatos *Amblyomma triste.* O ovário dessa espécie é do tipo panoístico, não possuindo, portanto, células nutridoras. Este órgão é composto por uma camada de células epiteliais que além de formarem a parede do ovário, também originam o pedicelo, estrutura que prende os ovócitos a sua margem externa, bem como pelos ovócitos. Em *A. triste*, os ovócitos passam por quatro estágios de desenvolvimento sincrônico, o que contraria os dados da literatura obtidos para outras espécies de carrapato. A classificação dos estágios do ovócito foi realizada baseada na presença de quatro características morfológicas: aspecto citoplasmático; localização da vesícula germinal; presença, quantidade e constituição dos grânulos de vitelo e presença de cório.

Abstract: This study presents the morphology, histology and the dynamics of vitellogenesis in females of the tick *Amblyomma triste*. The ovary in this species is of the panoistic type, therefore it lacks nurse cells. It is composed of a layer of epithelial cells that outwardly form the wall of the ovary, but also originate the pedicel, the structure that attaches the oocytes to its external margin, as well the oocytes themselves. In *A. triste*, the oocytes develop in four synchronic stages, which differs from the process in other tick species. The classification of the stages of the oocytes was carried out based on the presence of four morphologic characteristics: cytoplasm appearance; site of the germ vesicle; presence, quantity and constitution of the yolk granules and presence of chorium.

Key words: *Amblyomma triste;* tick; ovary; vitellogenesis; histology.

1. Introduction

Ticks are ectoparasites of great medical and veterinary importance as they cause huge blood loss in affected animals as well as acting as vectors for protozoa, virus, bacteria and other pathogens (Flechtmann, 1973). In this particular case, the tick species *Amblyomma triste* transmits *Rickettsia conorii* (Conti-Días, 2001) and *Rickettsia parkeri* (Venzal *et al*., 2004).

The tick species *A. triste* is distributed in the Neotropics and has been reported in Equador (Keirans, 1984), Argentina (Ivancovich, 1980), Uruguay and Brazil (Sinkoc *et al.,* 1997) infesting tapir (Kohls, 1956), dogs (Correa, 1954), capybara (*Hydrochaeris hydrochaeris)* (Sinkoc *et al.,* 1997), marsh deer *Blastocerus dichotomus* (Szabo *et al*., 2003) and humans (Venzal *et al*., 2003). It has also been reported also from opossum (*Didelphis marsupialis)* in an endemic area for Brazilian spotted fever in Pedreiras, State of São Paulo (Lemos *et al.,* 1997).

According to Till (1961), Sonenshine (1991), Denardi *et al.* (2004), Oliveira *et al.* (2005) and Saito *et al.* (2005), the reproductive system of Ixodidae ticks females consists of a horseshoe shaped tubular ovary located in the posterior region of the body, a pair of oviducts, a womb, a vagina and a genital opening.

There have been few studies of the reproductive system of ticks in general. The present study was designed to provide data on the morphology, and histology of the female reproductive system, and to describe the dynamics of vitellogenesis in *A. triste.*

2. Material and Methods

Semi-engorged females of *Amblyomma triste* ticks used in this study were from the tick colony maintained under controlled conditions (28 º C, 80% humidity and 12h photoperiod) at the Department of Animal Pathology, Veterinary College, UNESP – Jaboticabal, SP, Brazil. Equipment from the Histology and Electron Microscopy Laboratories of the Biology Department at

the Biosciences Institute, UNESP – Rio Claro, SP, Brazil, were utilized throughout the study.

Twenty-five specimens maintained in the refrigerator for thermal shock anesthesia were dissected in PBS solution (NaCl 7.5 g/L, Na2HPO4 2,38 g/L e KH2PO4 2,72 g/L). The ovaries were removed, sectioned and the oocytes were drawn using a camera lucida coupled to a Zeiss stereomicroscope.

2. 1. Scanning Electron Microscopy (SEM): The ovaries removed were fixed in Karnovsky solution for 24 hours, and dehydrated in a graded 70-100% ethanol and acetone series. The material were processed by critical point drying, sputtered with gold, and examined and photographed in a PHILLIPS 505 SEM.

2.2. Histology: The ovaries were fixed in 4% paraformaldehyde. The material was dehydrated in ethanol, embedded in JB4 resin for 24h at 4º C and transferred to plastic moulds previously filled with resin containing a catalyzer. After resin polymerization, the material was sectioned using a Sorvall JB4 microtome (Bio Red) and stained with hematoxylin and eosin, following routine histological procedures.

2.3. Histochemistry: Histochemical tests were applied in order to detect the presence of the following compounds: proteins (Bromophenol Blue according to Pearse [1985]); lipids (Nile Blue according to Lison [1985]); polysaccharides (PAS according to Junqueira & Junqueira [1983]).

3. Results

3.1. Ultramorphology

The scanning electron microscopy revealed the ovary of ticks *Amblyomma triste* as an single tubular organ, horseshoe shaped, with a large

number of oocytes attached at different stages of development. These oocytes develop simultaneously and synchronically along the ovary (Fig. 1A).

3.2. Histology

The ovary of the tick *Amblyomma triste* is classified as panoistic, consisting of only epithelial and germinative cells, without either specialized nurse or follicular cells.

The epithelial cells are small and cubic with rounded nuclei, forming the wall of the ovary and delimiting a narrow lumen (Fig. 2A).

In some regions of the epithelial wall of the ovary, the presence of the pedicel can be observed. This is a multicellular structure, whose primary function is to fix the oocytes, found at different stages of development, to the wall of the ovary (Fig. 2A).

3.3. Oocyte classification

The histology of oocytes of *A. triste* showed some particularities, regarding the presence, production and distribution of the cytoplasmatic formers. To better understand the descriptions, we subdivided the cytoplasm of the oocyte in three regions:

- Central region (Ce): occupying around 2/3 of the cytoplasm of the oocyte, and containing the germinal vesicle.

- Intermediate region (In): a narrow band located outside central region, but separated from the peripheral region.

- Peripheral region (Per): making close contact with the plasmic membrane. It should be emphasised that for each stage of development, these regions present special characteristics as described below.

Oocytes Stage I or in early stages of development – small cells (0.07mm), rounded, with germ vesicle or a nucleus occupying the greater part of the cytoplasm and containing an evident nucleolus.The cytoplasm is homogeneous without yolk granules (Figs. 1B; 2A).

Oocytes Stage II –larger than the previous ones (0.15mm), elliptical and the germ vesicle is situated near to the oocyte pole toward the pedicel. Thin homogeneous granulation is evenly scattered in the cytoplasm. Other histological characteristics are similar to those found in stage I (Figs. 1B; 2A,C).

Oocytes Stage III or intermediate stage – These are larger than those in the stages I and II (0.21mm), rounded and/or elliptical. The cytoplasm contains small yolk granules, probably of protein and polysaccharide, that mainly occupy the central region (Ce) and larger and lipidic granules, forming a band in the periphery (Per) of the oocyte. The germ vesicle and the nucleolus appear as in the previous stages (Figs. 1B; 2B).

Oocytes Stage IV – They are the largest oocytes (0.24mm) and possess a rounded morphology. The germ vesicle is observed occupying the region of the oocyte close to the pedicel. The cytoplasm has yolk granules constituted by several elements; these granules occupy different sites, that is, at the central region (Ce), there are small ones constituted by proteins and polysaccharides, at the periphery (Per), there are those of lipid nature, and at the intermediary region (In), there is a fine strip of large granules of protein nature. The plasmic membrane present initial characteristics of chorium once it is being already deposited. (Figs. 1B; 2D).

3.4. Histochemistry

3.4.1. Bromophenol Blue – protein detection.

This test reveals that the presence of protein in the ovary epithelial cells (Fig. 2E) as well as in different stages of development in its boundaries, in the nuclear envelope, and in the cytoplasm. The concentration of protein in the cytoplasm of the oocytes varied according to the stage of development (Table 1) and the nucleolus always reacted strongly.

3.4.2. Nile Blue – lipid detection.

This test reveals a moderate positivity in the cytoplasm and in the cytoplasmic limit of the ovary cell wall (Fig. 3A) and, the presence of lipid in all the present oocytes in the ovary (Table 1), having the cellular limit and the nuclear envelope showing moderate positivity and the cytoplasm has varied according to the involved region.

The nucleolus of the germ vesicle was evident in the oocytes, stained by hemotoxylin.

3.4.3. PAS – polysaccharide detection.

The ovary wall cells were weakly positive in their cytoplasm and cellular limits (Fig. 3D). In addition, the oocytes showed a gradual increase in the concentration of polysaccharide during their development (Table 1).

Weak positivity was observed in the limits of oocytes I, II and III, while the nuclear envelope was negative (Figs. 3D, F). On the other hand, the cellular limits possessed initial characteristics of chorium, being thicker and moderately positive in oocyte IV (Fig. 3E).

The nucleolus of the germ vesicle was evident in the oocytes, stained by hemotoxylin.

4. Discussion

The analysis of the ovary of adult *Amblyomma triste* ticks showed that it is a single structure, tubular, continuous and horseshoe-shaped. These data corroborate those obtained by Till (1961) in *Rhipicephalus appendiculatus*, and other tick species (Sonenshine 1991, Denardi *et al.* 2004, Oliveira *et al.* 2005, Saito *et al.* 2005.

In other species of ticks, oogenesis proceeds proximally, since the oocytes in early stages of development are in the distal region while the more developed are proximal (Denardi *et al.* 2004, Oliveira *et al.* 2005 and Saito *et al.*

2005.). The present study did not show the same disposition of oocytes in the ovary of *A. triste*, nor was there great morphologic variation between the oocytes. This suggests that, in *A. triste*, the development of the oocytes is synchronic.

The ovary of *A. triste* is of the panoistic type, as in *A. cajennense* (Denardi *et al*. 2004), *R. sanguineus* (Oliveira *et al*. 2005) and *Boophilus microplus* (Saito *et al*. 2005).

The ovary wall of *A. triste* consists of a single epithelium of small cells with rounded nuclei, delimiting a lumen and, by larger cells, the oocytes, in different stages of development, which are located on the external surface attached by a cellular pedicel, as observed by Till (1961) in *R. appendiculatus*, and in other species (Balashov 1983, Denardi *et al*. 2004, Oliveira *et al*. 2005 and Saito *et al*. 2005). *A. triste* does not have specialized nurse cells, confirming the findings in other species (Balashov 1983, Denardi *et al*. 2004, Oliveira *et al*. 2005 and Saito *et al*. 2005).

The histological and histochemical study of the oocytes of *A. triste* demonstrated significant differences, mainly related to their stage of development, and the constitution and sequence of deposition of yolk elements. These data permitted them to be classified into four stages (I to IV), based in that proposal of Denardi *et al.* (2004) for the *A. cajennense* oocytes, in which the authors have described the occurrence of five different developmental stages. The referred authors have detected large disposition of yolk granules, mainly of protein nature in the oocytes under stage IV and V. Oliveira *et al*. (2005) reported the process of five developmental stages despites some oocytes in undergoing resorption, in *R. sanguineus* and Saito *et al.* (2005), six developmental stages in the oocytes of *B. microplus.* Oliveira *et al*. (2005) and Saito *et al.* (2005) found large protein granules of yolk in the oocytes IV and V of *R. sanguineus* and *B. microplus,* respectively, what confirms elevated accumulation of yolk in these species of ticks during the vitellogenesis. The present study has not detected elevated yolk deposition in the *A. triste* oocytes.

The main characteristics for the classification of *A. triste* oocyte were: cytoplasmic appearance, location of the germ vesicle, presence, quantity and constituency of the yolk granules and presence of chorium.

In this way, stage I oocytes were classified due to its rounded form, homogeneous cytoplasm, constituted mainly by lipids, without yolk granulation and with germ vesicle occupying the central region. Stage II oocytes, are elliptic, with cytoplasm presenting thin proteic, lipidic and polysaccharidic granulation and germ vesicle displaced to the pole near to the fixation point of the oocyte in pedicel. Stage III oocytes, are rounded and/or elliptic, the cytoplasm presenting small yolk granules mainly of proteic and polysaccharidic nature in the central region (Ce) and larger lipidic in the peripheral region of the cell (Per). Stage IV oocytes, are rounded, with cytoplasm having small protein and polysaccharide granules mainly in the central region (Ce), larger lipid bodies in the peripheral region (Per) and larger protein granules in the intermediate region (In). Presence of chorium of glycolipoproteic nature is observed at stage IV.

According to Ramamurty (1968), in studies focused to insects, the yolk consists of lipids, proteins and polysaccharides and they would be deposited in this sequence, which could be either free or chemically bound forming complex of several natures. On the other hand, Balashov (1983) registered that the final products of the tick vitellogenesis process would be yolk granules constituted by proteins and lipids only. Through histochemical techniques, there was detected in females of *A. triste* a positive reaction for the three elements, highlighting the glycolipoproteic character of the yolk in this species, data that do not corroborate with the ones of Balashov (1983). However, the presence of protein, polysaccharide, and lipid yolk granules were already found by Denardi *et al.* (2004), Oliveira *et al.* (2005) and Saito *et al.* (2005) in other species of ticks.

Regarding to the deposition sequence of the yolk elements in the *A. triste* oocytes, the results here obtained confirm the sequence suggested by Ramamurty (1968) and by Oliveira *et al.* (2005), once in the oocytes I, there was detected the presence of lipids, and little or none element of protein and

polysaccharide natures; in the oocytes II, the quantity of proteins was higher than the polysaccharide ones; these were only found in significative quantity from the oocyte III. Concerning the oocytes IV, they presented elevated quantity of lipids and proteins and moderated quantity of polysaccharides.

In the oocytes IV, there is occurring the chorium deposition. In *A. triste*, the chorium is constituted by proteins, lipids and polysaccharides (glycolipoproteic), corroborating to the data of Denardi et al. (2004) and Oliveira et al. (2005) for other species of ticks and opposing to those obtained by Saito et al. (2005) in *B. microplus,* which suggest the lipoproteic nature of the chorium.

According to Camargo-Mathias and Caetano (1998), in the ant species *Neoponera villosa*, the chorium is comprised by proteins, lipids, and polysaccharides synthesized by follicular cells, in the case of meroistic polytrophic ovaries. Due to the absence of follicular cells in ticks, the chorium is produced by the oocyte through exocystic vesicle deposition (Balashov, 1983).

The chorium is responsible for preserving the oocyte structure and consequently the species (King, 1960), protecting the eggs against the mechanic shocks, avoiding desiccation and predation, protecting against the temperature oscillation and mainly promoting the gaseous exchange for the oxygenation of the embryo (Hinton, 1982).

In the present study, there was not observed oocytes under the stage V of development as described by Denardi *et al.* (2004) in *A. cajennense*. Furthermore, there was not dissected oocytes in undergoing resorption, which are characterized by the abnormal aspect, cellular disorganization, and autophagic vacuoles in the cytoplasm. According to Oliveira *et al.* (2005) and Saito *et al.* (2005) in *R. sanguineus* and in *B. microplus,* respectively, this process is frequent, however the causes are not known yet.

5. Acknowledgments

We thank to Gerson Mello Souza, Ronaldo del Vecchio, Cristiane Marcia Mileo and Paulo Aruanã Cezar for their technical support and, CAPES and

FAPESP (*Fundação de Amparo à Pesquisa do Estado de São Paulo*) for financial support (grant 2002/10.149-7).

Part of this work has been facilitated through the International Consortium of Ticks and Tick-Borne Diseases (ICTTD-3) supported by the European Union under contract number 510561.

6. Legend of Figures

Figure 1. A. Scanning Electron Microscopy **(SEM)** of *Amblyomma triste* ovary showing oocytes in several phases of development. **B.** Diagrammatic summary of oogenesis in ovarian cross-section of *Amblyomma triste.*
Ce= Central region; cl = cell limit; ch= chorium; ep= ovary epithelium; gv= germ vesicle; In= Intermediate region; moo= mature oocyte; nu= nucleolus; oo= young oocyte; ov= ovary; p= pedicel; Per= Peripheric region; **I**= Oocyte **I**; **II=** Oocyte **II**; **III=** Oocyte **III**; **IV=** Oocyte **IV**.
Bars: A= 0,1mm; B=0,05 mm.

Figure 2. Histological sections of *Amblyomma triste* ovary.
A. Stage I and **II** Oocytes stained by H-E. **B. Stage III** Oocyte stained by H-E. **C.** Detail of **stage II** oocyte stained by H-E. **D.** Detail of **stage IV** oocyte stained by H-E. **E. Stage I** Oocytes stained by Bromophenol Blue. **F.** Detail of **stage II** oocyte stained by Bromophenol Blue. **G.** Detail of **stage III** oocyte stained by Bromophenol Blue. **H.** Detail of **stage IV** oocyte stained by Bromophenol Blue.
ch= chorium; cl = cell limit; ep= ovary epithelium; gv= germ vesicle; nu= nucleolus; p= pedicel; arrow= yolk granules.
Bars: A,B= 0,05 mm; C,D= 0,02 mm; E = 0,05 mm; F, G, H = 0,02mm.

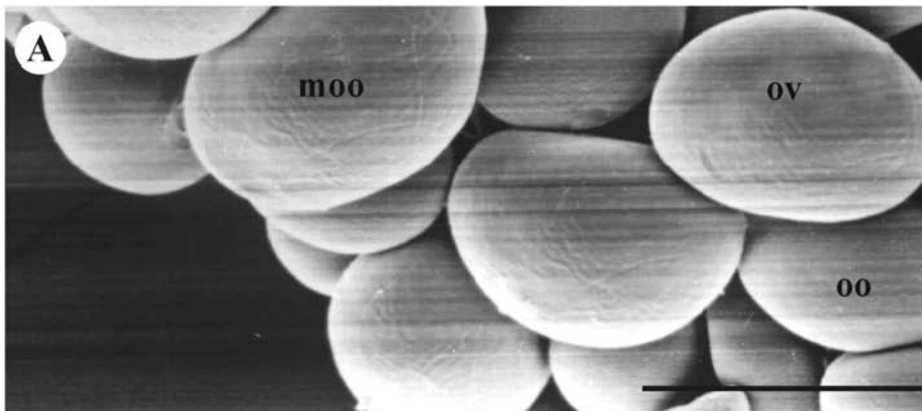
Figure 3. Histological sections of *Amblyomma triste* ovary.
A. Stage I and **II** Oocytes Oocytes stained by Nile Blue. **B. Stage II** and **III** Oocytes stained by Nile Blue. **C.** Detail of **stage IV** oocyte stained by Nile Blue.

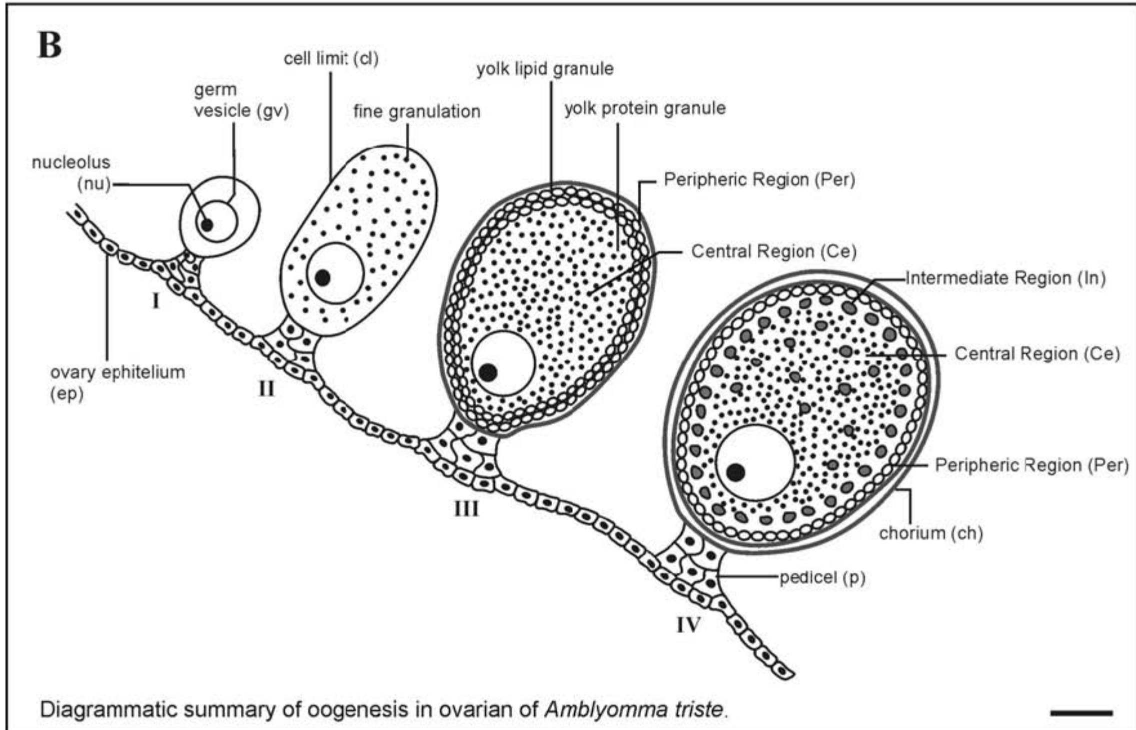
D. Stage I Oocytes stained by PAS. **E.** Detail of **stage IV** oocyte stained by PAS. **F. Stage II** and **III** Oocytes stained PAS.

cl= cell limit; ch= chorium; ep= ovary epithelium; gv= germ vesicle; nu= nucleolus; p= pedicel; arrow= yolk granules.

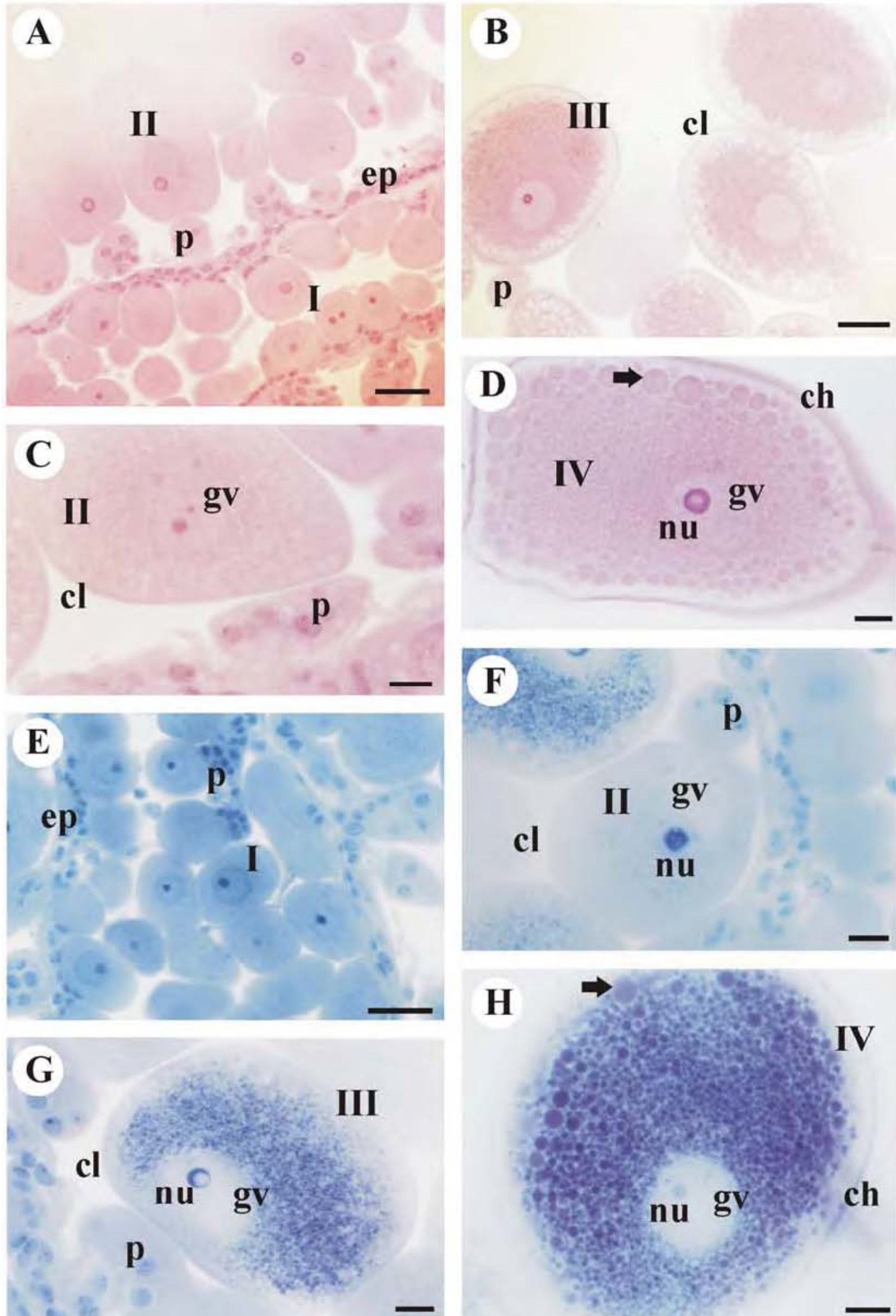
Bars: A,B,C,D= 0,05 mm; E= 0,02 mm; F = 0,05 mm.

1





2



3

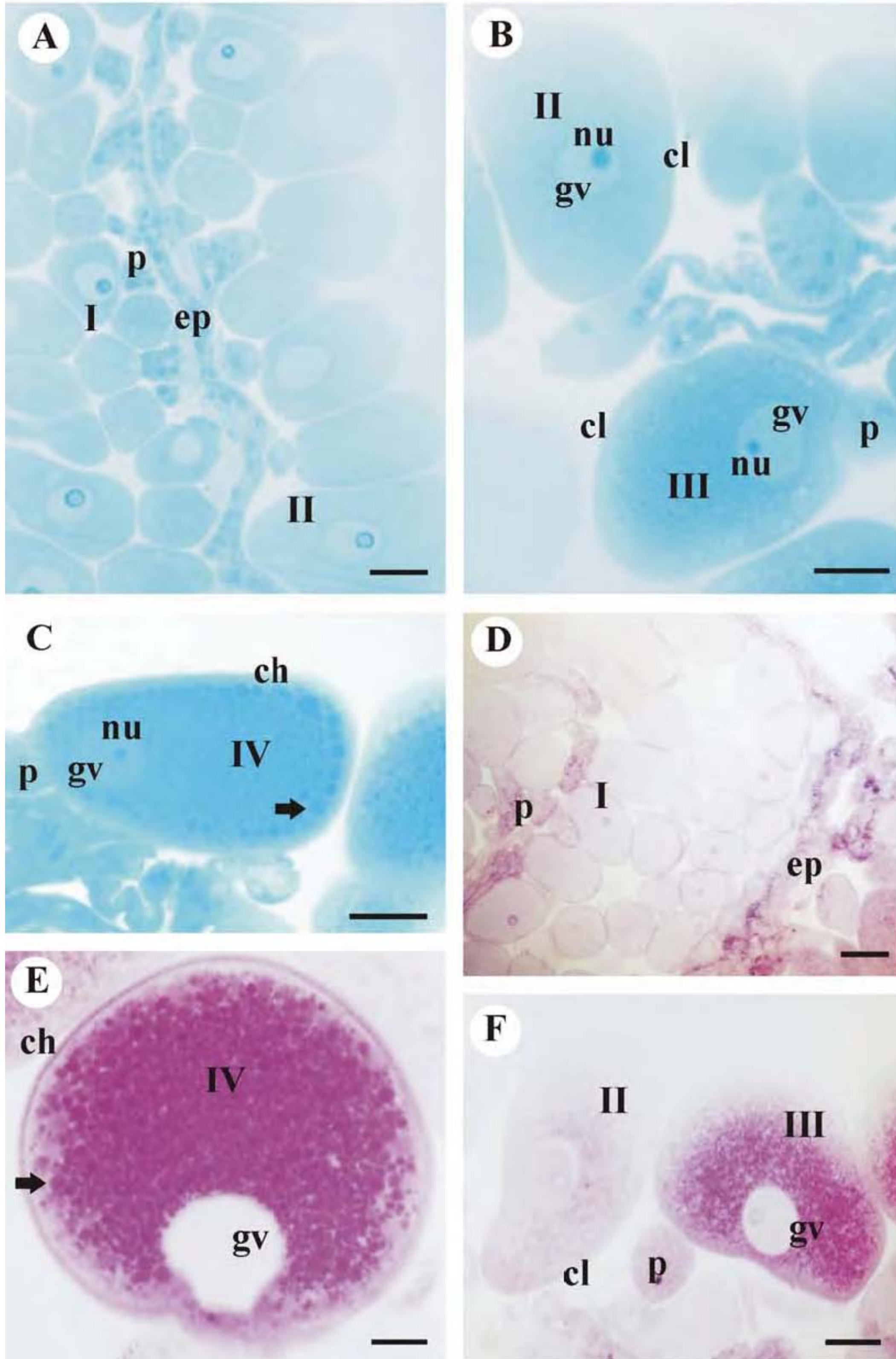


Table 1 –Histochemical results obtained from oocytes of the tick *Amblyomma triste*.

Oocytes Stages	Vitelline Elements	Germ vesicle	Central Region	Intermediate Region	Peripheral Region	Chorium
I	Proteins (Fig. 2E)	+	Cytoplasm++, no granules	Cytoplasm ++, no granules	Cytoplasm ++, no granules	___
	Lipids (Fig. 3A)	+	Cytoplasm++, no granules	Cytoplasm ++, no granules	Cytoplasm ++, no granules	___
	Polysaccharides (Fig. 3D)	___	Cytoplasm +, no granules	Cytoplasm +, no granules.	Cytoplasm +, no granules.	___
II	Proteins (Fig. 2F)	+	Thin granulation +	Thin granulation +	Thin granulation +	___
	Lipids (Figs. 3A, B)	+	Thin granulation +	Thin granulation +	Thin granulation +	___
	Polysaccharides (Fig. 3F)	___	Thin granulation +	Thin granulation +	Thin granulation +	___
III	Proteins (Fig. 2G)	+	Small granules, +++	Small granules, +++	Small granules, ++	___
	Lipids (Fig. 3B)	+	Small granules ++	Small granules, ++	Larger granules ++	___
	Polysaccharides (Fig. 3F)	___	Small granules, ++	Small granules, ++	Small granules, ++	___
IV	Proteins (Fig. 2H)	+	Small granules, +++	Larger granules, +++	Small granules, +++	+
	Lipids (Fig. 3C)	+	Small granules, +++	Small granules, +++	Larger granules, +++	+
	Polysaccharides (Fig. 3E)	___	Small granules,+++	Small granules,+++	Small granules,+++	++

8. References

Balashov, Y.S., 1983. The female reproductive system. In: Balashov, Y.S. (Ed.), An atlas of ixodid tick ultrastructure. Entomological Society of America, Russian, pp. 98-128.

Camargo-Mathias, M.I., Caetano, F. H., 1998. Ultrastructural study of the follicular epithelium in oocytes of *Pachycondyla* (*Neoponera) villosa* ants (Hymenoptera: Ponerinae). Biocell 22, 53-60.

Conti-Días, I. A., 2001. Rickettsiosis por *Rickettsia conoori* (fiebre botonosa del Mediterráneo o fiebre de Marsella). Estado actual en Uruguay. Revista Medica Uruguay 17, 119-124.

Correa, O., 1954. Carrapatos determinados no Rio Grande do Sul. Biologia, patologia e controle. Boletim da Diretoria de Produção Animal 10(18), 38-54.

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 *Amblyomma cajennense* (Acari: Ixodidae). Veterinary Parasitology 125, 379-395.

Flechtmann, C. H. W., 1973. Ácaros de importância médico-veterinária. Livraria Nobel S.A., São Paulo, pp. 55-65.

Hinton, H. E., 1982. Biology of Insect Egg Shells. Pergamon, Oxford, pp.80-100.

Ivancovich, J.C., 1980. Reclasificación de algunas especies de garrapatas del género *Amblyomma* (Ixodoidea) en la Argentina. Investigaciones Agropecuarias 15, 673-682.

Keirans, J.E., 1984. George Henry Faulkner Nuttall and the Nuttall tick catalogue. United States Department of Agriculture Research Series Miscellaneous Publications (1438), pp. 1610-1700

King, R.C., 1960. Oogenesis in adult *Drosophila melanogaster* IX. Studies on the cytochemistry and ultrastructure of developing oocytes. Growth. 24, 265-323.

Kohls, G.M., 1956. Concerning the identity of *Amblyomma maculatum, Amblyomma tigrinum, Amblyomma triste* and *Amblyomma ovatum* of Koch, 1844. Proceedings of The Entomological Society of Washington 58, 143-147.

Lemos, E.R.S., Machado, R.D., Coura, J.R., Guimarães, M.A., Freire, N.M., Amorim, M., Gazeta, G.S., 1997. Epidemiological aspects of the Brazilian spotted fever: seasonal activity of ticks collected in an endemic area in São Paulo. Revista da Sociedade Brasileira de Medicina Tropical 30, 181-185.

Oliveira, P.R., Bechara, G.H., Denardi, S.E., Saito, K.C., Nunes, E.T., Camargo-Mathias, M.I., 2005. Morphological characterization of the ovary and oocytes vitellogenesis of the tick *Rhipicephalus sanguineus* (LATREILLE, 1806) (Acari: Ixodidae). Experimental Parasitology 110, 146-156.

Ramamurty, P.S., 1968. Origin and distribution of glycogen during vitellogenesis of the scorpion fly *Panorpa communis*. Journal Insect Physiology 14, 1325-1330.

Saito, K. C., Bechara, G.H., Oliveira, P.R., Nunes, E.T., Denardi, S.E., Camargo-Mathias, M.I., 2005. Morphological, histological, and ultrastructural studies of the ovary of the tick *Boophilus microplus* (CANESTRINI, 1887) (Acari: Ixodidae). Veterinary Parasitology 129, 299-311.

Sinkoc, A.L., Brum, J.G.W., Müller, G., Begrow, A., Paulsen, R.M.M., 1997. Ixodideos parasitos de capivaras (*Hydrochaeris hydrochaeris* L., 1766) da Estação Ecológica do Taim, Rio Grande, RS. Ciência Rural 27 , 119-122.

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

Szabó, M. P. J., Labruna, M. B., Pereira, M. C., Duarte, J. M. B., 2003. Ticks (Acari: Ixodidae) on Wild Marsh-Deer (*Blastocerus dichotomus*) from Southeast Brazil: Infestations before and after habitat loss. Journal of Medical Entomology 40 , 268 – 274.

Till, W.M., 1961. A contribution to the anatomy and histology of the brown ear tick *Rhipicephalus appendiculatus*. Swets & Zeitlinger Publishers, Amsterdam, pp. 65-97.

Venzal, J.M., Guglielmone, A.A., Estrada-Pena, A., Cabrera, P.A., Castro, O., 2003. Ticks (Ixodida: Ixodidae) parasitising humans in Uruguay. Annals of Tropical Medicine & Parasitology 97, 769-772.

Venzal, J.M, Portillo, A., Estrada-Pena, A., Castro, O., Cabrera, P.A., Oteo, J.A., 2004. *Rickettsia parkeri* in *Amblyomma triste* from Uruguay. Emerging Infectious Diseases 10, 1493-1495.

Capítulo 2:

Título do artigo: Female ticks of *Amblyomma triste* (Koch, 1844) (Acari: Ixodidae). Ovaries ultrastructural analysis.

Autores: Patrícia Rosa de Oliveira; Maria Izabel Camargo Mathias e Gervásio Henrique Bechara.

Periódico: Micron.

Situação: Submetido e aceito.

Resumo: O presente estudo apresenta a análise ultra-estrutural do ovário de carrapatos *Amblyomma triste*. O ovário dessa espécie é do tipo panoístico, assim não possui células nutridoras nem foliculares. O ovário é composto por uma camada de células epiteliais, que formam a sua parede e que são pobres em organelas apresentando somente algumas mitocôndrias em seu citoplasma e, pelos ovócitos, os quais estão presos na margem externa desta parede epitelial por meio de um pedicelo multicelular. Os ovócitos foram classificados em quatro estágios diferentes de desenvolvimento. Ovócitos I possuem córtex homogêneo. Ovócitos II apresentam pequenos grânulos de vitelo com morfologia e conteúdo variados. Ovócitos III possuem grânulos de vitelo ($\mathbf{p_1}$) e mitocôndrias que apresentam desorganização de suas cristas e de suas membranas. Ovócitos IV possuem grânulos protéicos de vitelo sob duas formas: $\mathbf{p_1}$ e $\mathbf{p_2}$, e inclusões lipídicas. Todos os ovócitos apresentam microvilos em sua membrana plasmática. Os resultados obtidos permitiram sugerir que além da produção exógena de elementos vitelínicos, existe também a produção endógena, muitas vezes ocorrendo simultaneamente, e contribuindo para o desenvolvimento e crescimento desses ovócitos.

Abstract: The present study presents the ultrastructural analysis of the ovary of ticks *Amblyomma triste*. The ovary of this specie is of the panoistic type, not presenting nurse and follicular cells. The ovary is composed of a layer of epithelial cells, that form its wall and that are poor in organelles, only presenting some mitochondrias in their cytoplasm and, of oocytes, which remain attached to the external margin of this epithelial wall by a multicellular pedicel. The oocytes were classified in four different developmental stages. Oocytes I possess homogeneous cortex. Oocyte II presents small yolk granules with varied morphology and content. Oocyte III possess yolk granules (**p1**) and mitochondrias that present disorganization of their crests and membranes. Oocyte IV possess yolk proteic granules under two forms: $\mathbf{p_1}$ and $\mathbf{p_2}$, and lipid droplets All the oocytes present microvilli in the plasmic membrane. The obtained results allowed suggest that beyond the exogenous production of yolk

elements, also can exists an endogenous production, both simultaneously, and contributing for the development and growth of these oocytes.

Key words: *Amblyomma triste*; tick; ovary; vitellogenesis; ultrastructure***.***

1. Introduction

The economical importance of ticks is widely acknowledge and is related to their feeding habits. When feeding, many species of ticks transmit diseases to man and other animals are caused by protozoa, viruses, Rickettsias and spirochetes (REY, 1973).

Numerous studies are currently under way aiming at finding an efficient control strategy that would minimize the damages caused by these parasites. A new tick control perspective is the immunological one, consisting in the identification, isolation, and synthesis of proteins that protect the tissues and organs of the tick, mainly those of the reproductive system, with the objective of obtaining a vaccine (TELLAM et al., 1992; WILLADSEN, 1997).

According to Till (1961), Sonenshine (1991) and Said (1992), the female reproductive system of ticks generally consists of a large U-shaped ovary located at the posterior region of the body, with a pair of oviducts, an uterus, a muscular connection tube, vagina, and genital opening.

Due to absence of data on the morphology and physiology of the female reproductive system of ticks *Amblyomma triste*, the present work has aimed to study the ovary ultrastructure, complementing the morpho-histologic aspects that will assist in the comprehension of the vitellogenesis process and in the possible control of this species of ticks.

2. Material and Methods

Semi-feeding females of *A. triste* used in this study were from the tick colony maintained in controlled conditions (28 º C, 80% humidity and 12h photoperiod) at the Department of Animal Pathology, Veterinary College, UNESP – Jaboticabal, SP, Brazil. Equipment from the Electron Microscopy Laboratories of the Biology Department at the Biosciences Institute, UNESP – Rio Claro, SP, Brazil, were utilized throughout the study.

Twenty-five specimens maintained in the refrigerator for thermal shock anesthesia were dissected in saline solution (NaCl 7.5 g/L, Na_2HPO_4 2,38 g/L and KH_2PO_4 2,72 g/L).

2. 1. Transmission Electron Microscopy (TEM)

The material was fixed in 2.5% glutaraldehyde, postfixed in 1% OsO_4, and embedded in Epon Araldite. Then it was embedded in pure Epon resin and polymerized at 60º C for 72 hours. Ultrathin sections were contrasted with uranyl acetate and lead citrate. Afterwards, screens containing ultrathin sections of the material were observed and photographed in a PHILLIPS 100 TEM.

3. Results

The ovary wall of the tick *Amblyomma triste* is constituted of small epithelial cells that in determined regions proliferate and form the pedicel, a structure that attaches the oocytes in different stages of development, to the ovary wall (Fig. 1A).

The cells of this epithelium are small and pavimentous. The large and oval nucleus occupies the greater part of the cytoplasm and that is restricted to a thin layer to its contour. The cytoplasm is poor in organelles, only presenting some mitochondria (Fig. 1A). A thick basal lamina moderately electrondense supports this epithelium. The cells are connected to each other by means of interdigitations of their lateral membranes (Fig. 1A).

Ultrastructurally, it is observed that the oocytes I possess homogeneous cortex with few organelles, as ribosomes, free or adhered to the membrane of the rough endoplasmic reticulum, which shows little developed and under lamellar form (Fig. 1C). However it is detected large amount of mitochondria with varied shapes: elliptical, rounded or elongated (Figs. 1B, C). They are relatively small and occupy preferentially the region around of the germ vesicle, which is located in the center of the oocyte I, with rounded morphology,

dispersed chromatin and a large nucleolus with two distinct regions: one granular and one fibrous (Fig. 1B).

Limiting the content of the oocyte I, it is detected the electrondense plasmic membrane with some microvilli (Fig. 1D) mainly toward the pedicel and the basal lamina that appears subdivided in two layers: a more internal and thicker layer, which is in direct contact with the microvilli, and a more external and thinner layer of fibrillar aspect. In the region between the internal portion of the basal lamina and the plasmic membrane of the oocyte can be observed vesicles containing material being transported to the oocyte I (Fig. 1D).

Oocytes II are larger than ones of stage I. The cytoplasm begins to present small yolk granules with varied morphology and content, located uniformly all over the cytoplasm. Granules of lipid constitution are electronluscents; already the proteic granules possess larger electrondensity (Figs. 2A, B).

As much as the organelles, the mitochondria are those finding more frequently, with varied shape and distributed all over the cytoplasm. Also is observed the presence of free ribosomes, some myelinic bodies and lamellar and vesicular rough endoplasmic reticulum, both little developed (Figs. 2A,B).

The oocytes II present larger amount of microvilli in their plasmic membrane, which also are supported in a thick basal lamina subdivided in the portions internal and external. Vesicles and electrondense material between the plasmic membrane and the basal lamina are detected (Fig. 2C).

In oocytes III is still observed the germ vesicle (Fig. 2D). In this stage the oocytes presents numerous mitochondria mainly rounded by all over the cytoplasm. Some of them possess individual characteristics as disorganization of their crests and membranes. Myelinic bodies, Golgi complex and lamellar and vesicular rough endoplasmic reticulum also are observed (Figs. 2E, F).

In the cytoplasm also yolk granules are found, that were called here by **p1**, they are homogeneous, with foam aspect, moderately electrondense and probably present proteic constitution, as well as electronluscent lipid droplets (Figs. 2E, F).

In these oocytes, still are observed microvilli in the plasmic membrane, which together with the thick basal lamina remain as in the previous stages (Figs. 2D, F).

In oocytes IV the germ vesicle now with sufficient irregular morphology is still observed, many reentrances in its envelope, little heterochromatin as well as accumulation of small granules strongly electrondense and rRNA *(nuage)* to its round (Figs. 3A, B).

In these oocytes are found many yolk granules of varied constitution and with different electrondensity. Free or grouped electronluscent lipid droplets occupy mainly regions near to the periphery (Per) (Figs. 3E; 4A, B, D). Nevertheless the granules of proteic constitution occupy all the cytoplasm and present themselves under two forms: as moderately electrondense and homogeneous granules (**p1**) and, as granulation of strong electrondensity, less frequent, that probably, form glyco or lipoproteic complexes, called here by **p2** (Figs. 3C, E; 4A, B). Mitochondria, lamellar rough endoplasmic reticulum little developed and Golgi complex are present (Figs. 3C, E; 4A, B, C, D).

In the peripheral region of the oocyte IV it is noticed the presence of microvilli longer than those found in the previous stages (Figs. 3C, D) and a larger space between the plasmic membrane and basal lamina. In this place between these structures it is being deposited the chorium through endocytic vesicles.

4. Discussion

The application of the techniques of transmission electron microscopy confirmed the classification of the ovaries of the tick *Amblyomma triste* as panoistic type, where all cells present correspond to oogonia or future oocytes. This classification was based on the same one that was adopted by Denardi *et al.* (2004) in ticks *A. cajennense*, Oliveira *et al.* (2005), in *R. sanguineus* and Saito *et al.* (2005), in *B. microplus*.

The stage of development of the oocytes was identified according to the proposal by Oliveira *et al. (in press)*, where the main characteristics considered

were the aspect of the cytoplasm, the localization of the germ vesicle, the presence, amount and constitution of yolk granules and the presence of the chorium.

Oocytes I presented homogeneous cortex with many mitochondria and without yolk granules. Oocytes II showed small yolk granules, larger amount of mitochondria, however, little rough endoplasmic reticulum, which appears under the form of cisternum of low electrondensity.

In the beginning of the development of the oocytes (stage I) of the *A. triste*, the mitochondria are located around the germinal vesicle. According to Balashov (1983), these organelles are associated with aggregates of riboproteins disposed under the form of granules and fibrils. The related author affirms that the function of this association of the mitochondria still is unknown.

Subsequently, the mitochondria have been found all over the cytoplasm of oocytes, pointing out to a preparation of these cells for the period of exogenous incorporation of the vitellogenesis, in which these organelles become prerequisite for the active transportation of material (Balashov, 1972).

In the peripheral region next to the pedicel from the oocytes I and II, small vesicles and microvilli are found, what confirms that in these cells are occurring high incorporation rate of extra-ovarian elements for the composition of yolk, which would be being synthesized for cells of pedicel and incorporated by oocytes through pinocytic vesicles.

Balashov (1983) described the presence of microvilli in the surface of the oocytes in growth of *Hyalomma asiaticum* and this study corroborates his datas. These structures would be related with the increase of the surface of the oocytes necessary for bigger efficiency of the active transport of elements provided the exogenous sources to the oocyte (Balashov, 1983).

According to Balashov (1983), the exogenous sources of yolk elements production in ticks, would be located in their intestine cells. However, Sonenshine (1991) suggested the participation of fat body in ticks vitellogenesis, in which the cells would secrete great amounts of the vitellogenic protein in hemolymph, which would, in turn, be taken up by developing oocytes via endocytosis. The present study still suggests the participation of cells of the

pedicel in the vitellogenesis process, synthesizing and providing substances to the interior of the oocytes.

In oocytes III many microvilli in the plasmic membrane were found and larger number and size of yolk granules, however low amount of rough and smooth endoplasmic reticulum, organelles responsible for the endogenous synthesis of lipids and proteins, respectively. This allows us to suggest that in oocytes III exists larger evidence that the elements of yolk come from extra-ovarians sources, but also does not exclude the possibility that it is occurring endogenous production of these elements.

With relation to the lipids and according to Camargo-Mathias (1993), in studies with ants *Neoponera villosa*, the mitochondria would be one of the origin of this element in the oocytes of these insects, confirming the data of other authors (Bonhag, 1958; Wigglesworth, 1964). Ranade (1933) affirmed previously that the mitochondria would be transformed in lipid yolk bodies and suggested that the lipid material in the oocytes would come from breaking and destruction of the mitochondrial crests. Oocytes III of *A. triste* presented modifications in their mitochondria, such as the disorganization of their crests and membranes beyond the presence of lipids and low amount of smooth endoplasmic reticulum as already mentioned above. The present study suggests, then, that lipid components could have the mitochondria as their source, as well as exogenous source of this element.

In oocytes IV it is verified the presence of numerous yolk granules of varied constitution and electrondensity and medium size, of many microvilli in their plasmic membrane as well as the deposition of chorium in the region between the basal lamina and the plasmic membrane through vesicles of exocytosis. The permanence of the microvilli until the last stage of development of the oocytes of the *A. triste* suggests that the incorporation of extra-ovarians elements is continuous during all the vitellogenesis process and that occurs in superior amount to the endogenous production of yolk.

The germ vesicle in oocytes IV presents many reentrances as well as nuclear pores that allows the passage of material from the nucleus to the cytoplasm, probably rRNA. Consequently, around of the germ vesicle, is

occurring the increase in the amount of ribosomes, formation of the *nuage* and the deposition of small electrondense granules, such as was observed in *A. triste*. These structural characteristics can indicate the occurrence of processes of endogenous synthesis of yolk still in oocytes IV. The passage of material through the nuclear pores also was observed in the oocytes from the *Atya scabra* by Cruz-Landim (1997).

Diehl (1970) affirms that the yolk proteins of the ticks are immunologically identical to proteins of hemolymph. This could be evidence that it can also exist some extra-ovarians sources of elements for the formation of yolk of the oocytes. Second the related author other tissues could synthesize these substances that would be transported by hemolymph, taken up by microvilli and incorpored through pinocytic vesicles.

Balashov (1983) suggested that in ticks *H. asiaticum*, the yolk would have dual origin, endogenous and exogenous. However, related author affirms that the endogenous production occurs before exogenous production, what not happen with *A. triste*, since oocytes in initial stages of development (I and II) already possess morphologic evidences that would confirm the transport of elements come from the exterior to the interior of the oocyte, such as: microvilli in the plasmic membrane and great amount of mitochondria in the contact region oocyte/cells of pedicel.

Denardi *et al.* (2004), in oocytes of the *A. cajennense* and Oliveira *et al.* (2005), in *R. sanguineus* described a fifth (V) stage of development, which presents great proteic granules and chorium subdivided in two layers: the endochorium (more internal and electrondense) and the exochorium (more external and less electrondense), nevertheless such characteristics had not been detected in no oocyte of the tick *A. triste*. In this species, the chorium is thinner than that found for the related authors for other species of ticks and without subdivisions.

5. Acknowledgments

We thank Miss Cristiane Marcia Mileo, Miss Monika Iamonte, Mr. Antônio Teruyoshi Yabuki and Mr. Ronaldo Del Vecchio for their technical support and CAPES for financial support.

Part of this work has been facilitated through the International Consortium of Ticks and Tick-Borne Diseases (ICTTD-3) Coordination Action financed by the INCO Program of the European Comission (Project No.510561).

6. Legend to Figures

Figure 1A. Ultrastructure showing epithelium cells (**ep**) of ovary of *Amblyomma triste*. **B.** General view of oocyte **I**. **C**. Detail of the cytoplasm near of the germ vesicle of oocyte **I**. **D.** Peripheric region of oocytes **I**.

bl = basal lamina; **pm =** plasmic membrane; **n** = nuclei; **ne =** nuclear envelope; **m** = mitochondria; **gv =** germ vesicle; **nu =** nucleoli; **lrer** = lamellar rough endoplasmic reticulum; **mv =** microvilli; **arrow =** interdigitations of the lateral membranes.

Bars: A= 2 μm; B= 2 μm; C= 2 μm; D= 2 μm

Figure 2 A. General view of oocyte **II (II). B.** Detail of the central cytoplasm of oocytes **II. C** Peripheric region of oocytes **II**. **D.** General view of oocyte **III** (**III**). **E.** Detail of the central cytoplasm of oocytes **III**. **F.** Peripheric region of oocytes **III**.

bl = basal lamina; **l =** lipid granule; **lrer** =lamellar rough endoplasmic reticulum; **mv =** microvilli; **m** = mitochondria; **n =** nuclei; **p =** pedicel; **pm** = plasmic membrane; $\mathbf{p_1}$ **=** yolk proteic granules p_1; **vrer** = vesicular rough endoplasmic reticulum; **gv =** germ vesicle; **g =** Golgi complex.

Bars: A= 10 μm; B= 2 μm; C= 1 μm; D= 10μm; E= 2 μm; F=2 μm.

Figure 3 A. Cytoplasm near of the germ vesicle of oocyte **IV**. **B.** Detail of the cytoplasm near of the germ vesicle of oocyte **IV**. **C**. Peripheric region of oocytes **IV**. **D**. Detail of the peripheric cytoplasm of oocytes **IV**. **E.** Central cytoplasm of oocytes **IV**.

ch = chorium; **g** = Golgi complex; **gv =** germ vesicle; **l** = lipid granule; **m** = mitochondria; **mv =** microvilli; **ne =** nuclear envelope; **lrer** = lamellar rough endoplasmic reticulum; **pm** = plasmic membrane; $\mathbf{p_1}$ **=** yolk proteic granules p_1; $\mathbf{p_2}$ **=** yolk proteic granules p_2; **vrer** = vesicular rough endoplasmic reticulum; **head arrow** = granules and *nuage* around the germ vesicle.

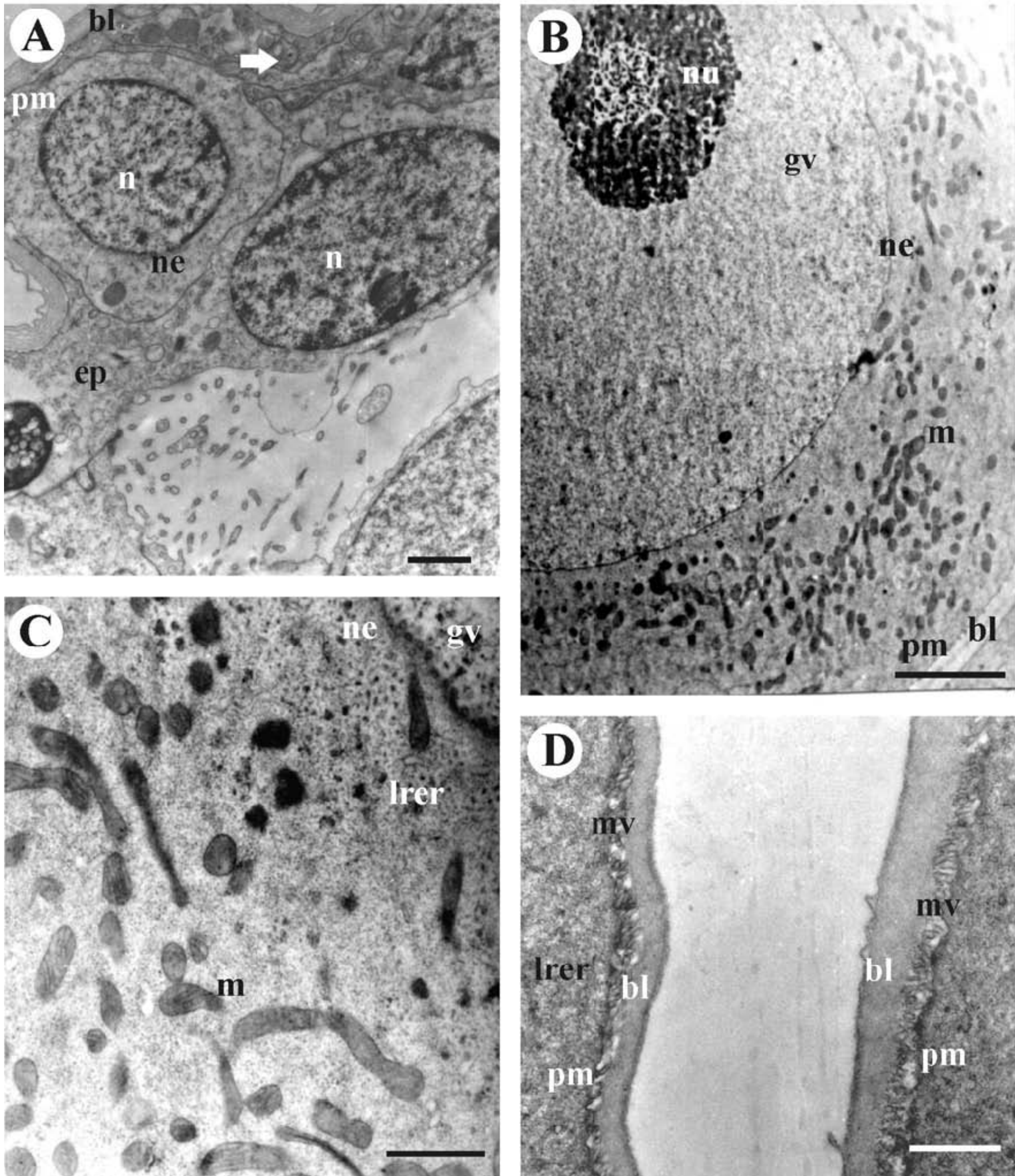
Bars: A= 2 μm; B= 1 μm; C= 1 μm; D= 1 μm; E= . 2 μm.

Figure 4 A. Cytoplasm of oocytes **IV**. **B.** Detail of the central cytoplasm of oocytes **IV**. **C.** Detail of the intermediary cytoplasm of oocytes **IV**, showing yolk proteic granules. **D.** Detail of the peripheric cytoplasm of oocytes **IV**, showing lipid granules.

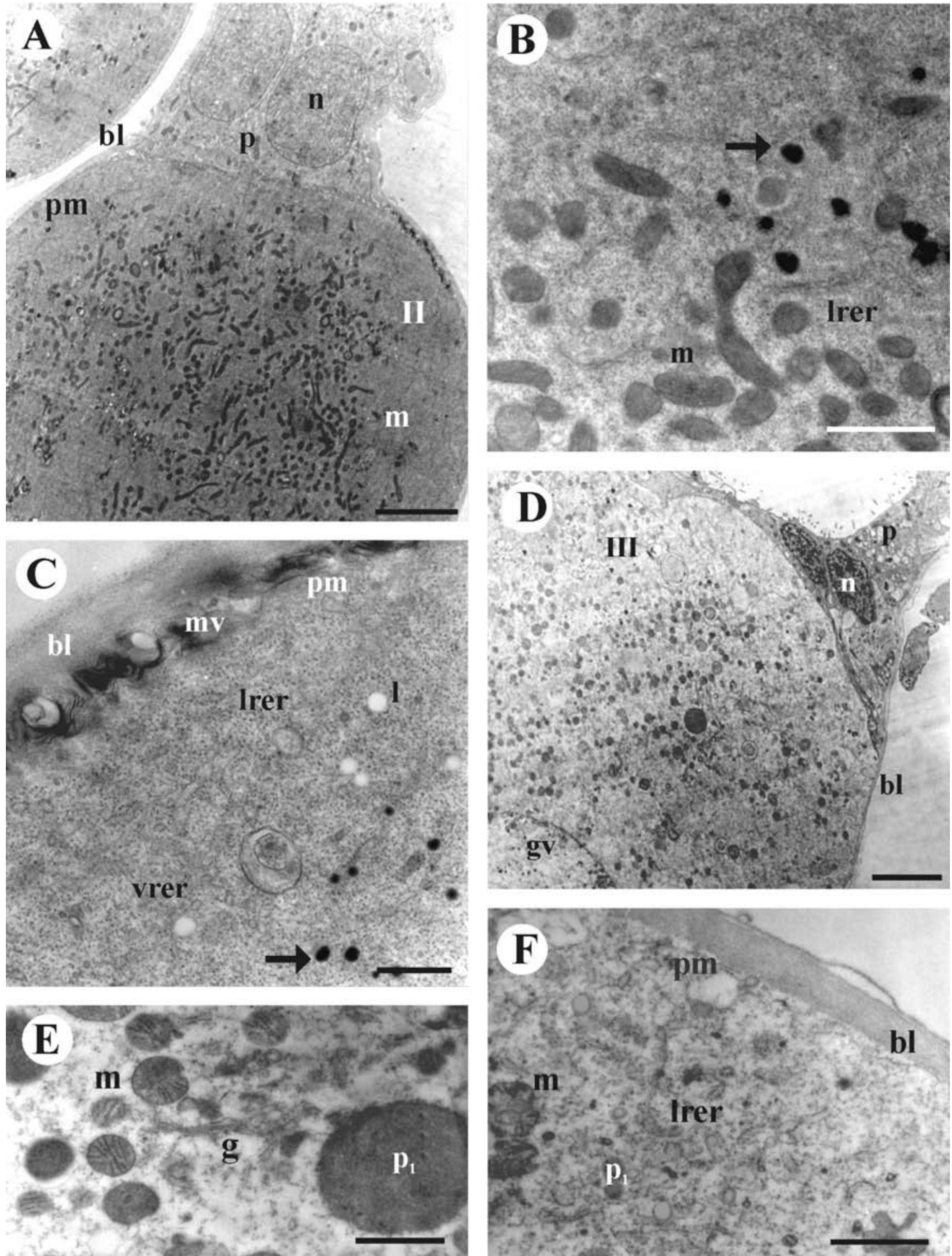
m = mitochondria; **l** = lipid granule; **lrer** = lamellar rough endoplasmic reticulum; $\mathbf{p_1}$ **=** yolk proteic granules p_1; $\mathbf{p_2}$ **=** yolk proteic granules p_2; **vrer** = vesicular rough endoplasmic reticulum**.**

Bars: A= 1 μm; B= 1 μm; C= 1 μm; D= 1 μm.

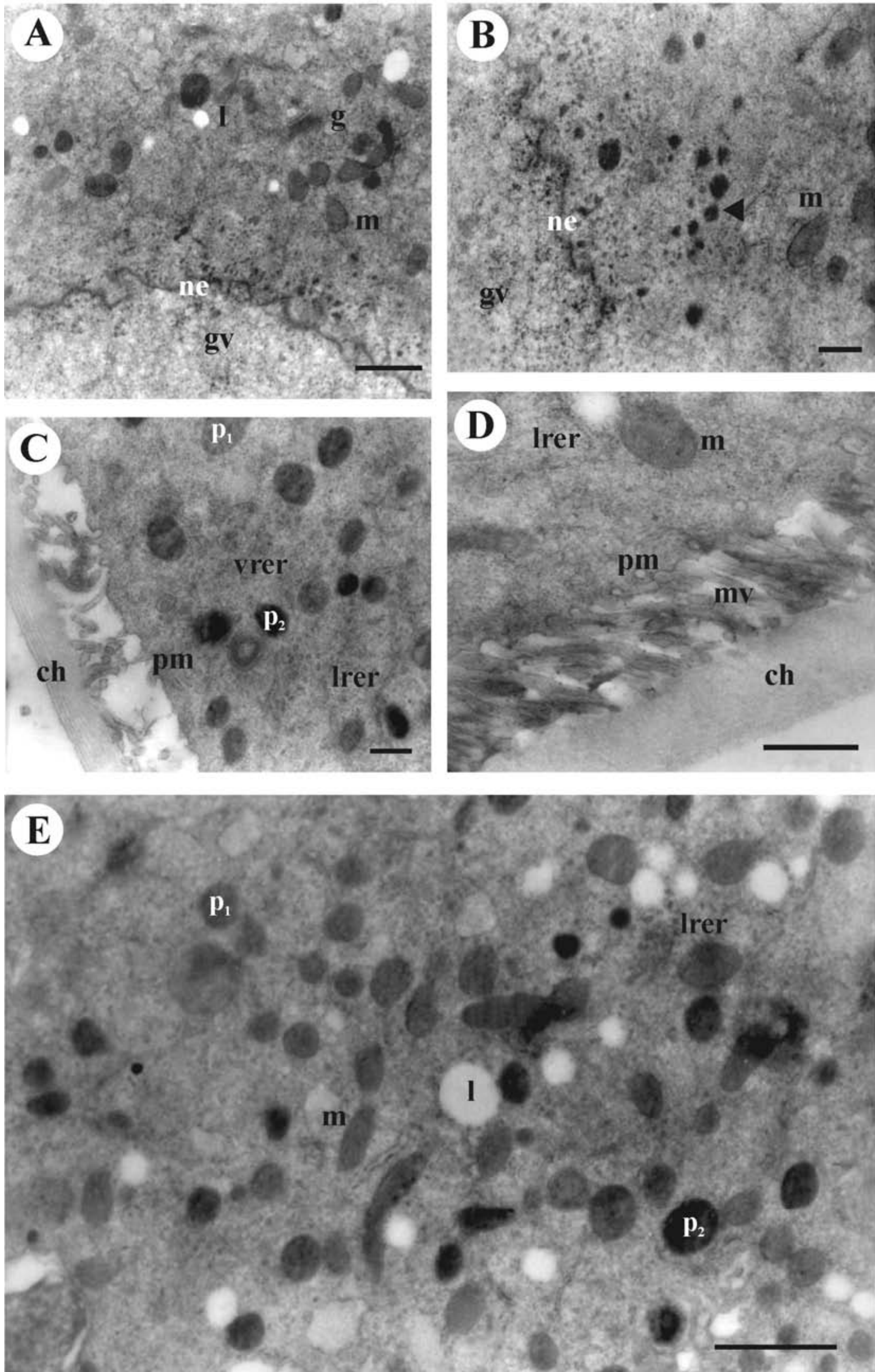
1



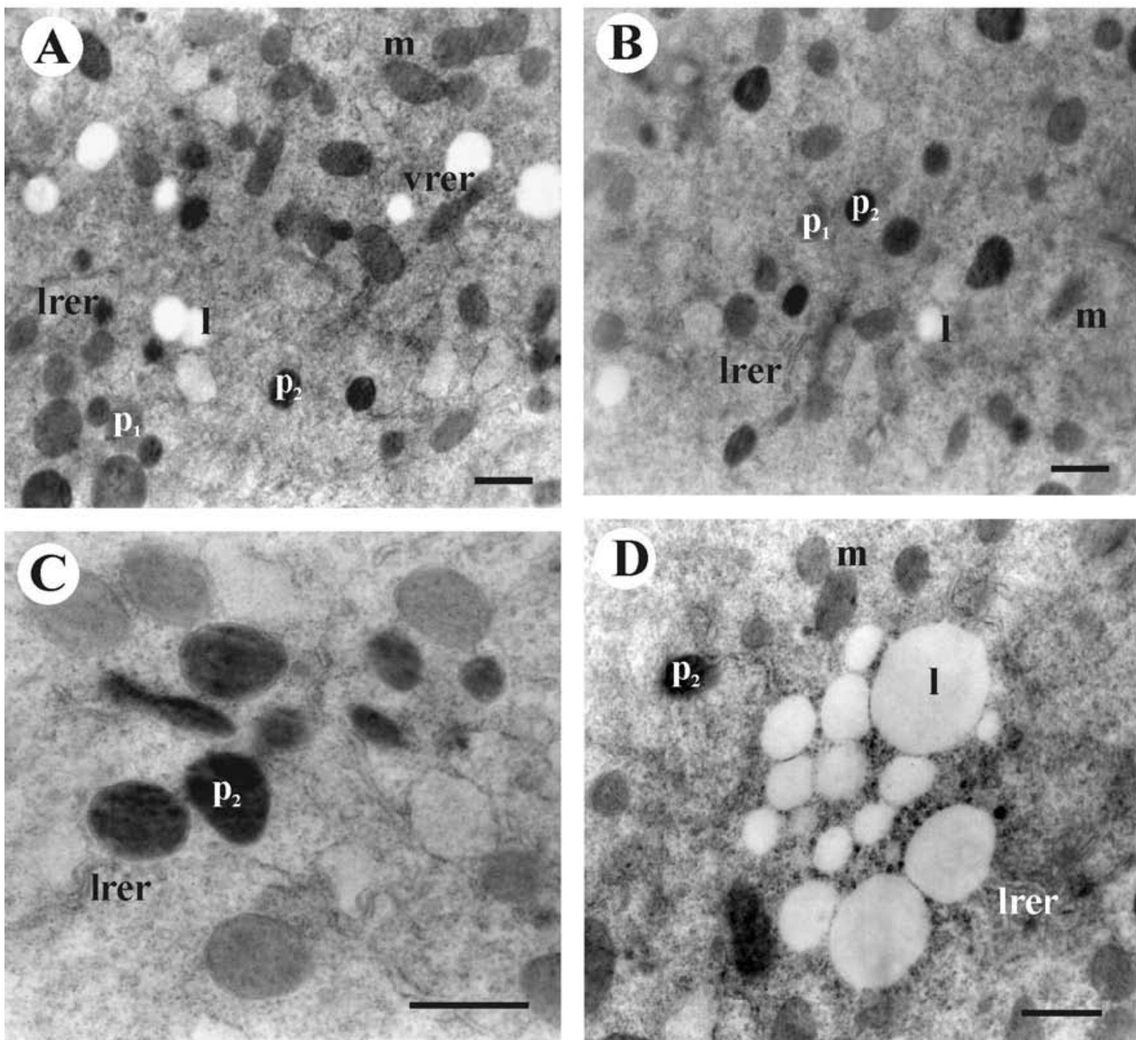
2



3



4



7. References

Balashov, Y.S., 1972. A translation of bloodsucking ticks (Ixodidae) vectors of diseases of man and animals. Miscellaneous Publications of the Entomological Society of America 8, 159--376.

Balashov, Y.S., 1983. The female reproductive system. In: BALASHOV, Y.S. (Ed.), An atlas of ixodid tick ultrastructure. Entomological Society of America, Russian, pp. 98--128.

Bonhag, P. F., 1958. Ovarian structure and vitellogenesis in insects. Annual Review of Entomology 3, 137--160.

Cruz-Landim, C., 1997. Cell reorganization and cell death during the secretory cycle of the hypopharyngeal gland in Meliponinae bee workers (Hymenoptera: Apidae). Acta Microscopica 6, 75--78.

Camargo-Mathias, M.I., 1993. Histoquímica e ultra-estrutura dos ovários de operárias e rainhas de formigas *Neoponera villosa* (Hymenoptera: Ponerinae). Tese (doutorado em Zoologia), Instituto de Biociências,Universidade Estadual Paulista, Rio Claro, 156p.

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 *Amblyomma cajennense* (Acari: Ixodidae). Veterinary Parasitology 125, 379--395.

Diehl, P. A., 1970. Zur Oogenese bei *Ornithodoros moubata* (Murray) (Ixodoidea: Argasidae) unter besonderer Berücksichtigung der Vitellogenese. Acta Tropical 27, 301--355.

Oliveira, P.R., Bechara, G.H., Denardi, S.E., Saito, K.C., Nunes, E.T., Camargo-Mathias, M.I., 2005. Morphological characterization of the ovary and oocytes vitellogenesis of the tick *Rhipicephalus sanguineus* (LATREILLE, 1806) (Acari: Ixodidae). Experimental Parasitology 110, 146--156.

Oliveira, P.R., Bechara, G.H., Camargo-Mathias, M.I., 2005. Females Ticks of *Amblyomma triste* (Kock, 1844) (Acari: Ixodidae). Ovaries description and vitellogenesis process. Experimental Parasitology, in press.

Ranade, V., 1933. On the cytoplasmic inclusions in the oogenesis of *Periplaneta Americana*. Linn. Allahad. University Science Bulletin 9, 85--121.

Rey,L., 1973. Parasitologia. Guanabara Koogan, São Paulo, 212p.

Said, E.A., 1992. A contribution to the anatomy and histology of the female reproductive system of *Amblyomma cajennense* (Acarina : Ixodidae). Journal of the Egyptian Society of Parasitology 22, 385--393.

Saito, K. C., Bechara, G.H., Oliveira, P.R., Nunes, E.T., Denardi, S.E., Camargo-Mathias, M.I., 2005. Morphological, histological, and ultrastructural studies of the ovary of the tick *Boophilus microplus* (CANESTRINI, 1887) (Acari: Ixodidae). Veterinary Parasitology 129, 299--311.

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

Tellam, R.L., Smith, D., Kemp, D. H., 1992. Vaccination against ticks. In: Yong, W. K. (Ed.), Animal parasite control using biotechnology. CRC Press, Boca Raton, pp. 303--331.

Till, W.M., 1961. A contribution to the anatomy and histology of the brown ear tick *Rhipicephalus appendiculatus*. Swets & Zeitlinger Publishers, Amsterdam, pp. 65-97.

Wigglesworth, V. B., 1964. The hormonal regulation of growth and reproduction in insects. In: Beament, J. W. L., Theherne, J. E., Wigglesworth, V. B. (Eds), Advances in Insect Physiology. Academic Press, New York, pp. 247--336.

Willadsen, P., 1997. Novel vaccines for ectoparasites. Veterinary Parasitology 71, 209--222.

Capítulo 3:

Título do artigo: Vitellogenesis in the tick *Amblyomma triste* (Koch, 1844) (Acari: Ixodidae). Determining a new role for pedicel cells.

Autores: Patrícia Rosa de Oliveira; Maria Izabel Camargo Mathias e Gervásio Henrique Bechara.

Periódico: Experimental Parasitology.

Situação: Submetido.

Resumo: O presente estudo apresenta novos dados sobre o processo da vitelogênese de carrapatos *Amblyomma triste.* O ovário dessa espécie é composto por uma parede de células epiteliais, por células germinativas que darão origem aos ovócitos e pelo pedicelo, estrutura celular, que além de fixar os ovócitos à margem externa da parede epitelial, também sintetiza e fornece substâncias que são captadas pelos ovócitos e que auxiliarão no desenvolvimento dos mesmos. No presente trabalho, a função do pedicelo como fornecedor de elementos para os ovócitos durante a vitelogênese fica confirmada nessa espécie de carrapatos.

Abstract: This study presents new data on the vitellogenesis of the tick *Amblyomma triste*. The ovary of this species consists of a wall of epithelial cells, germ cells that will develop into oocytes, and the pedicel, a cellular structure that besides to attach the oocytes to external interface of the epithelial wall, also synthesizes and provides substances to oocytes that will promote their development. In this study, the role of the pedicel as a provider of compounds for oocytes during vitellogenesis is described for this tick species.

Key words: *Amblyomma triste*; tick; ovary; vitellogenesis; ultrastructure; pedicel.

1. Introduction

Ticks are considered parasites of vertebrate animals and can feed on a variety of hosts and even humans. Throughout their biological cycle, they can survive long periods without a host, sheltered in the vegetation and crevices in the soil (Walker, 1994).

Amblyomma, a genus of the Ixodidae family, includes species with long capitulum and the second segment at least twice the length of the width. They also present a pair of simple eyes, marginal festoons, and a decorated scutum. Approximately 30 species can found in Brazil (Rey, 1973), and among them, *Amblyomma triste.*

The ovary of Ixodidae ticks consists of a layer of epithelial cells forming the ovary wall, the pedicel attaching oocytes to the epithelial layer, as well as oocytes, which undergo several developmental stages (Till, 1961; Denardi *et al.*, 2004; Oliveira *et al.*, 2005; Saito *et al.*, 2005).

Due to the few studies performing the internal morphology of ticks in general, including *Amblyomma triste,* this study aims at providing more information on the ultrastructural aspects about the function of the cells forming the pedicel, showing their role in synthesizing and providing yolk elements to oocytes during vitellogenesis, as previously hypothesized in the literature.

2. Material and Methods

Semi-engorged females of *A. triste* ticks used in this study were obtained from a tick colony maintained under controlled conditions (28 º C, 80% humidity and 12h photoperiod) at the Department of Animal Pathology, Veterinary College, UNESP – Jaboticabal, SP, Brazil. Equipments from the Electron Microscopy Laboratories of the Biology Department at the Biosciences Institute, UNESP – Rio Claro, SP, Brazil, were utilized throughout the study.

Twenty-five specimens maintained in the refrigerator for thermal shock anesthesia were dissected in saline solution (NaCl 7.5 g/L, Na_2HPO_4 2.38 g/L and KH_2PO_4 2.72 g/L).

2.1. Transmission Electron Microscopy (TEM)

The material was fixed in 2.5% glutaraldehyde, postfixed in 1% OsO_4, and embedded in Epon Araldite. Ultrathin sections were contrasted with acetate and lead citrate. The material was then embedded in pure Epon resin and polymerized at 60º C for 72 hours. Afterwards, screens containing ultrathin sections of the material were observed and photographed in a PHILLIPS 100 TEM.

3. Results

The ovary wall of the tick *Amblyomma triste* consists of small epithelial cells. In certain areas these cells proliferate and form the pedicel, a structure that maintains oocytes of different developmental stages attached to the ovary.

In stage I and II oocytes, which here are considered in early stages of development, the pedicel consists of cylindrical cells with large elliptic nuclei occupying most of the cytoplasm, little heterochromatin, and several nuclear pores, suggesting cellular synthetic activity (Figs. 1A, B, C).

Regarding organelles, pedicel cells attaching stage I and II oocytes present numerous round mitochondria, as well as lamellar and vesicular rough endoplasmic reticulum occupying large portions of the cytoplasm (Figs. 1A, B, C).

The basal lamina supporting the plasmic membrane of pedicel cells (I and II) is thick and subdivided into two regions: internal and external, the latter exhibits a fibrillar and thinner structure. The basal lamina not only supports pedicel cells, but also the oocytes in stage I and II (Figs. 1A, C, D). However, is not inserted in the interface oocyte/pedicel cells. It externally encloses the complex as a whole. In the contact region stages I and II oocytes/pedicel cells, membrane specializations the similar to microvilli are present in both cell types, as well as large quantity of vesicles, indicating an intense exchange of material between cells (Figs. 1A, C).

Other cells of the pedicel, but that are not in direct contact with stage I and II oocytes, present lateral membrane interdigitations increasing the adhesion among them (Fig. 1B).

When the oocyte reaches stage II of its development, pedicel cells attaching it to the ovary differ from those attaching stage I oocytes by exhibiting the first signs of ultrastructural changes, characterized by loss of cytoplasmic integrity, that is, the beginning of cytoplasmic fragmentation (Figs. 1D, E).

The morphology of pedicel cells in contact with stage III oocytes changes from cylindrical to squamous, the nucleus becomes smaller, flat and irregular in shape. Pores present in the nuclear envelope are less frequent than in cells attaching oocytes in early developmental stage (I and II) (Fig. 2C).

Pedicel cells attaching stage III oocytes do not exhibit microvilli on the plasmic membrane in the entire oocyte/pedicel contact area. When present, this membrane specialization is limited to regions far from this contact area (Fig. 2A).

Mitochondria are not observed in the cytoplasm of these cells with the same frequency as in previous stages. Also, rough endoplasmatic reticulum is rarely observed (Figs. 2B, C). Several small vacuoles are detected. In this stage, these cells are already in the beginning of a degeneration process (Fig. 2B).

Pedicel cells attaching stage IV oocytes present, depending on location (cells near the ovarian epithelium and those near stage IV oocytes), different characteristics. The former, cells not in contact with oocytes, present flat nuclei with irregular shape and few nuclear pores. Few organelles are observed in their cytoplasm, only some small mitochondria (Figs. 2E, F). The plasmic membrane is thin and with no microvilli, similar to the observed in pedicel cells attaching stage III oocytes (Figs. 2E, F). These pedicel cells still exhibit lateral interdigitations increasing the adhesion among them (Figs. 2E, F). In pedicel cells in direct contact with stage IV oocytes, the nucleus gradually decreases in size, acquiring pycnotic characteristics. Organelles involved in cellular synthesis and mitochondria are no longer observed in the cytoplasm. Large autophagic vacuoles, however, are observed containing various components,

confirming that degeneration processes are in progress in these cells (Fig. 2D). The plasmic membrane of both types of pedicel cells attaching stage IV oocytes present the same characteristics, a thin plasmic membrane with no specializations.

4. Discussion

The ovary of the tick *Amblyomma triste* consists of an epithelial wall formed by small cells and oocytes. The latter are larger cells in different developmental stages attached to the ovary wall by a pedicel, a multicellular structure, as observed by Till (1961) in *R. appendiculatus,* Balashov (1983) in *Hyalomma asiaticum,* Denardi *et al.* (2004) in *A. cajennense,* Oliveira *et al.* (2005), in *R. sanguineus* and Saito *et al.* (2005), in *B. microplus*.

This study on *A. triste* ticks shows that in this species, the pedicel is also the result of the proliferation of epithelial cells of the ovary wall, as observed by Till (1961) in *R. appendiculatus.*

Ultrastructural analysis of pedicel cells of *A. triste* revealed that when observed attaching stage I and II oocytes, all these cells are cylindrical and exhibit characteristics of cells in synthetic activity, that is, large nucleus with many pores perforating the envelope, in addition to dispersed chromatin, abundant rough endoplasmic reticulum and mitochondria, as observed by Denardi *et al.* (2004) for *A. cajennense* and by Oliveira *et al.* (2005) for *R. sanguineus.*

Oliveira *et al.* (in press), studying the vitellogenesis process of *A. triste*, found in their ovary, stage I oocytes exhibiting cytoplasm without yolk granules and stage II oocytes with small granules. These authors, however, did not observe in stage II oocytes, the presence of organelles responsible for the synthesis of yolk compounds, that is, smooth and rough endoplasmatic reticulum. These data suggested that in stage II oocytes, the synthesis of yolk elements might occur also in pedicel cells (exogenous synthesis) that would then be transported into the oocyte. This would explain the presence of abundant rough endoplasmatic reticulum observed in the pedicel cells

attaching stage II oocytes of *A. triste*. In addition to rough endoplasmatic reticulum, some authors report the participation of the Golgi complex in the process of yolk synthesis. This organelle, however, was not observed in the pedicel cells of *A. triste*, indicating that it does not participate in the vitellogenesis in this species. Norrevang (1968), analyzing ultrastructural characteristics of the vitellogenesis in several animal groups, suggested that the Golgi complex would not be an organelle frequently observed and that few information would be obtained regarding its function and participation in the vitellogenesis of animals in general.

The large number of mitochondria present in the cytoplasm of pedicel cells attaching stage I and II oocytes of *A. triste* to the ovary indicates a strong evidence that these organelles might be involved in the active transport of yolk elements from the pedicel to the oocyte. This is confirmed also by the large amounts of microvilli in the cell membrane of pedicel cells in contact with these oocytes, which are interdigitated with microvilli present in their plasmic membranes, increasing the contact area among pedicel cells and oocyte.

Another observation supporting the participation of pedicel cells in providing yolk elements to oocytes in *A. triste* is the presence of electron dense vesicles being transported from the pedicel to oocytes. These vesicles seem to be transported especially near the contact area of the complex oocyte/pedicel, utilizing the energy produced by the several mitochondria concentrated in this region, characterizing it as active transport. Therefore, these characteristics confirm that in this species of tick, the pedicel is not only a structure attaching the oocyte to the ovary, but also it supports their growth.

The basal lamina supports the plasmic membrane of the oocyte as well as the pedicel, with exception of the contact region between the two cell types in ovaries of *A. triste*, as observed in *A. cajennense* (Denardi *et al.*, 2004), in *R. sanguineus* (Oliveira *et al.*, 2005) and in *B. microplus* (Saito *et al.*, 2005). According to Reddy and Locke (1990), the basal lamina would act as a barrier between compounds from the hemolymph while oocytes would monitor and select the traffic of macromolecules in both directions. This study also presents

data supporting this role, as we observed vesicles between the plasmic membrane and the basal lamina of oocytes of *A. triste.*

Pedicel cells attaching stage III oocytes of *A. triste* become squamous, exhibit smaller and flat nuclei, less pores in the nuclear envelope, reduced amounts of cytoplasmic organelles and several small vacuoles. This indicates that these cells begin to change as oocytes develop. The changes characterizing the onset of a degeneration process are first observed in pedicel cells in contact with stage III oocytes. This cellular degeneration is also observed in nurse cells of meroistic polytrophic ovaries commonly found and described for insects, especially Hymenoptera (Camargo-Mathias, 1993). In this Arthropod group, the role of nurse cells is to synthesize and send yolk elements to the oocyte, as we have shown for the pedicel cells of tick ovaries. In meroistic ovaries, the degeneration of nurse cells progresses as the oocyte reaches maturity until complete degeneration (Camargo-Mathias, 1993). Therefore, our study once again confirms the role of the pedicel as a source of yolk elements of tick oocytes.

The pedicel cells attaching stage IV oocytes of *A. triste* present distinct morphological characteristics depending on their location in the pedicel itself, that is, those distant from the oocyte or near the ovary wall, and those in direct contact with the stage IV oocyte. In the latter situation, pedicel cells exhibit pycnotic nuclei, nonorganized cytoplasm, and large vacuoles, and absence of microvilli in the plasmic membrane in contact with the oocyte. These modifications in pedicel cells indicate the end of vitellogenesis and consequently the end of transport of compounds to the oocyte, suggesting that membrane specializations, represented by microvilli, is one of the structures responsible for the transport of exogenous elements from the pedicel cells into oocytes. Also, at this stage, the pedicel has completed its function of producing and providing elements to oocytes, and then undergoes a gradual degeneration process. However, until the oocyte is mature enough to be released into the ovary lumen, the pedicel will continue to attach it.

Charniaux-Cotton and Payen (1985) reported in *Panulirus* that the retraction of the plasmic membrane and consequent absence of specializations would only occur at the end of the vitellogenesis for this species.

Till (1961), in previous studies, mentioned that several changes occur in the ovarian epithelium and pedicel cells in ovaries at the end of vitellogenesis in *R. appendiculatus.* The epithelium becomes thinner, the lumen dilates and stores large quantities of secretion acting as a lubricant. The pedicel cells, then, enter in a process of vacuolization (degeneration). In *A. triste*, vacuolization is also observed in the cytoplasm of these cells especially in those in direct contact with stage IV oocytes (final vitellogenesis stage), as observed by Till (1961), indicating once more that the pedicel enters into a gradual degeneration process at the end of the yolk synthesis.

Some authors believed that the pedicel cells were only responsible for attaching the oocytes to the ovary wall (Diehl, 1970; Brinton and Oliver, 1971). Aeschlimann and Hecker (1967), however, studying *Ornithodoros moubata*, first suggested a possible role of the pedicel in the process of synthesis and supply of yolk to support oocyte growth. The present study determines the role of the pedicel as a provider of yolk elements to tick oocytes.

5. Acknowledgments

We thank to Miss Monika Iamonte, Mr. Antônio Teruyoshi Yabuki, Ronaldo del Vecchio and Paulo Aruanã Cezar for their technical support and, CAPES and FAPESP (*Fundação de Amparo à Pesquisa do Estado de São Paulo*) for financial support (grant 2002/10.149-7).

Part of this work has been facilitated through the International Consortium of Ticks and Tick-Borne Diseases (ICTTD-3) supported by the European Union under contract number 510561.

6. Legends of Figures

Figure 1A. B. C. D. E. Ultrastructure of the pedicel cells of ovary of *Amblyomma triste* that are attaching the oocytes I and II. **A.** Contact region oocyte I (**I**)/pedicel cells. **B.** Detail of contact among pedicel cells (arrow), in the stage I. **C**. Detail of contact between oocyte I (**I**)/pedicel cells. **D.** General view of oocyte II (**II**) and pedicel. **E.** Detail of pedicel cells, in the stage II.

bl = basal lamina; **lrer** = lamellar rough endoplasmic reticulum; **m** = mitochondria; **mv** = microvilli; **n** = pedicel cells nuclei; **ne =** nuclear envelope; **p=** pedicel; **pm =** plasmic membrane; **vrer** = vesicular rough endoplasmic reticulum.

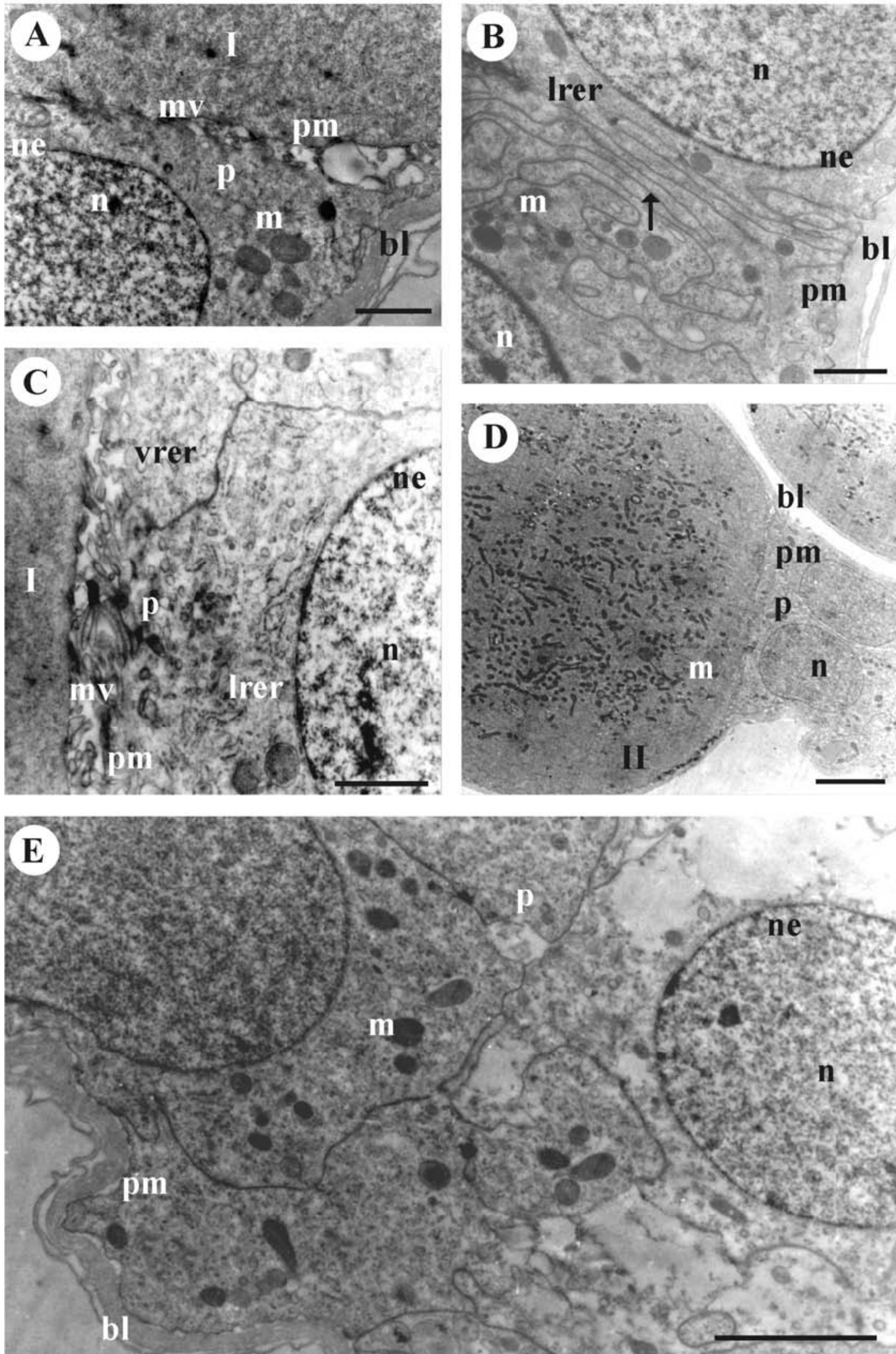
Bars: A**=**2 µm, B=2 µm, C=2 µm, D=10 µm, E=5µm.

Figure 2A. B. C. D. E. Ultrastructure of the pedicel cells of ovary of *Amblyomma triste* that are attaching the oocytes III and IV. **A.** General view of oocyte IIII (**III**) and of pedicel. **B**. Detail of the cytoplasm of pedicel cells, in the stage III. **C**. Detail of the pedicel cells nuclei, in the stage III. **D**. Contact region oocyte IV (**IV**)/pedicel cells (head arrow). **E.** General view of pedicel cells far from of the oocyte IV. **F.** Detail of the pedicel cells far from of the oocyte IV.

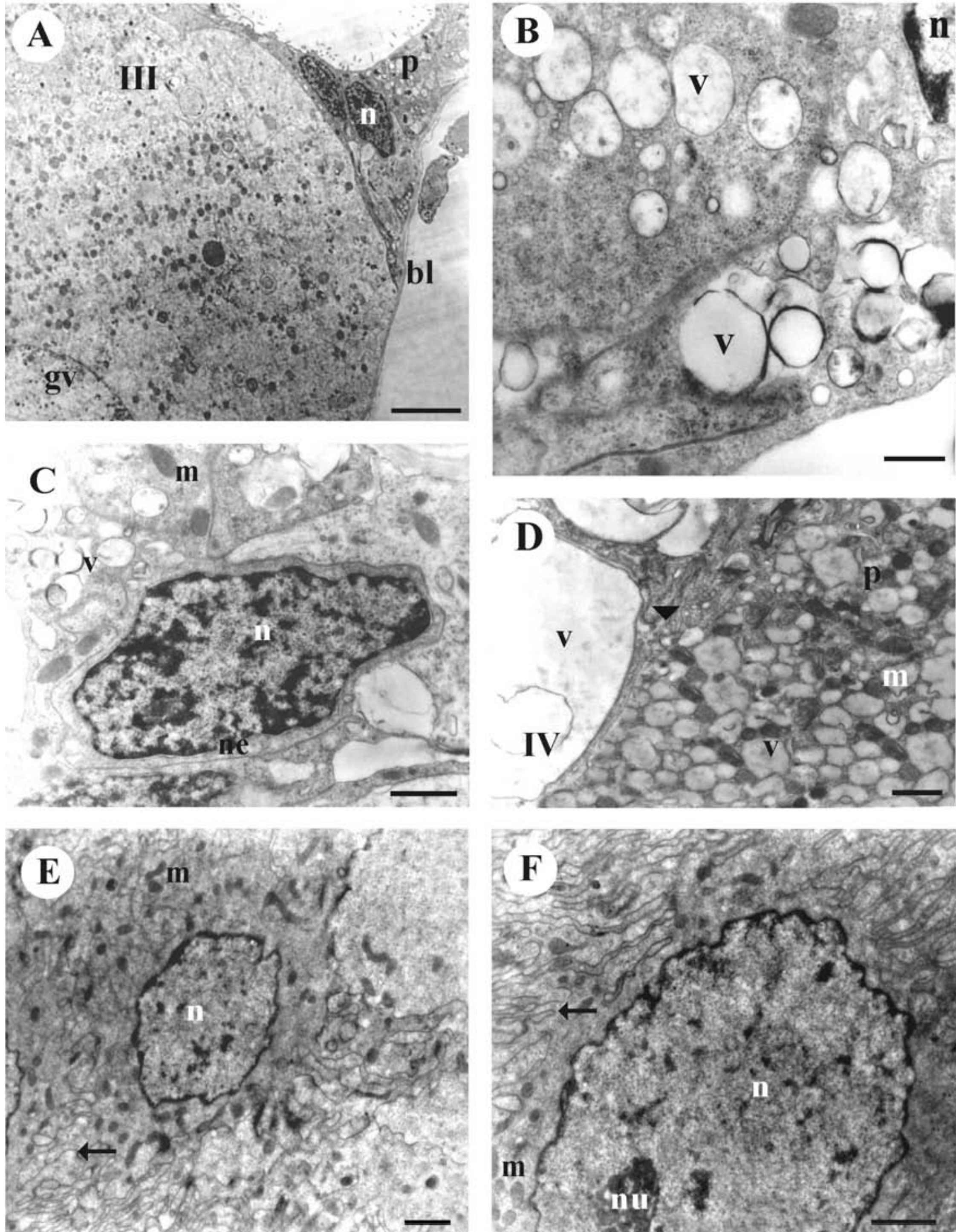
bl = basal lamina; **gv** = germ vesicle; **m** = mitochondria; **n** = pedicel cells nuclei; **ne =** nuclear envelope; **nu =** nucleoli**; p=** pedicel; **v** = vacuoles; **arrow** = membrane invagination of the pedicel cells, in stage IV; **head arrow** = contact region oocyte IV (**IV**)/pedicel cells.

Bars: A= 10 µm; B=1 µm; C= 2 µm; D= 2 µm; E=10 µm.

1



2



7. References

Aeschlimann, A., Hecker, H., 1967. Observations preliminaires sur l'ultrastructure de l'ovocyte en developpement chez *Ornithodoros moubata* Murray (Ixodidae: Argasidae). Acta Tropical 25, 225-243.

Balashov, Y.S., 1983. The female reproductive system. In: Balashov, Y.S. (Ed.), An atlas of ixodid tick ultrastructure. Entomological Society of America, Russian, pp. 98-128.

Brinton, L. P., Oliver, J. H. JR., 1971. Fine structure of oogonial and oocytes development in *Dermacentor andersoni* Stile (Acari: Ixodidae). Journal of Parasitology 57, 720-747.

Camargo-Mathias, M.I. 1993. Histoquímica e ultra-estrutura dos ovários de operárias e rainhas de formigas *Neoponera villosa* (Hymenoptera: Ponerinae). Instituto de Biociências – Universidade Estadual Paulista, Rio Claro, 156p.

Charniaux-Cotton, H., Payen, G., 1985. Crustacean Reproduction. In: Laufer, H., Downer, R. G. (Eds.), Endocrinology of selected invertebrate types. Alaw and Lius, New York, pp.279-300.

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 *Amblyomma cajennense* (Acari: Ixodidae). Veterinary Parasitology 125, 379-395.

Diehl, P. A., 1970. Zur Oogenese bei *Ornithodoros moubata* (Murray) (Ixodoidea: Argasidae) unter Besonderer Berücksichtigung der Vitellogenese. Acta Tropical 27, 301-355.

Norrevang, A., 1968. Electron microscopic morphology of oogenesis. International Review of Cytology 23, 114-186.

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

Oliveira, P.R., Camargo-Mathias, M.I., Bechara, G.H., 2005. Female Ticks of *Amblyomma triste* (Koch, 1844) (Acari: Ixodidae). Ovaries ultrastructural analysis. Micron, in press.

Reddy, J. T., Locke, M., 1990. The size limited penetration of gold particles through insect basal laminae. J. Insect Physiol. 36 (6), 397-399.

Rey,L., 1973. Parasitologia. Guanabara Koogan, São Paulo, 212p.

Saito, K. C., Bechara, G.H., Oliveira, P.R., Nunes, E.T., Denardi, S.E., Camargo-Mathias, M.I., 2005. Morphological, histological, and ultrastructural studies of the ovary of the tick *Boophilus microplus* (Canestrini, 1887) (Acari: Ixodidae). Veterinary Parasitology 129, 299-311.

Till, W.M., 1961. A contribution to the anatomy and histology of the brown ear tick *Rhipicephalus appendiculatus*. Swets & Zeitlinger Publishers, Amsterdam, pp. 65-97.

Walker, A., 1994. Arthropods of domestic animals. A guide to preliminary identification. Chapman & Hall, London, 5-60.

Capítulo 4:

Título do artigo: Ultrastructural detection of proteins, lipids and carbohydrates in oocytes of *Amblyomma triste* (Koch, 1844) (Acari; Ixodidae) during the vitellogenesis process.

Autores*:* Patrícia Rosa Oliveira; Amanda Juliana Ricardo; Maria Izabel Camargo Mathias e Gervásio Henrique Bechara.

Periódico: Experimental Parasitology.

Situação: Submetido.

Resumo: O ovário de carrapatos *Amblyomma triste* é do tipo panoístico, caracterizado pela presença apenas de ovogônias, com células nutridoras e foliculares ausentes. O presente estudo demonstrou que os ovócitos em todos os estágios de desenvolvimento (I – IV) estão presos ao ovário pelo pedicelo e que essa estrutura contribui para o fornecimento de carboidratos, lipídios e proteínas para os ovócitos durante o processo de vitelogênese. Os lipídios são depositados em todos os estágios dos ovócitos, distribuindo-se livremente e/ou, como observado nos ovócitos I, II, III e IV, formando complexos com outros elementos. As proteínas também são depositadas em todos os estágios dos ovócitos, no entanto, sua concentração diminui no estágio IV. Ocorre deposição de carboidratos a partir de ovócitos II, bem como nos ovócitos III e IV. O presente trabalho demonstrou que o vitelo nos ovócitos de *A. triste* tem natureza glicolipoproteíca e que os elementos são depositados na seguinte seqüência: primeiramente os lipídios e proteínas e finalmente os carboidratos.

Abstract: The ovary of tick *Amblyomma triste* is classified as panoistic, which is characterized by the presence oogonia without nurse and follicular cells. The present study has demonstrated that the oocytes in all developmental stages (I – IV) are attached to the ovary through a pedicel, which is a supportive structure for the carbohydrate, lipids, and proteins supplies for the oocytes during the vitellogenesis process. The lipids are deposited during all oocyte stages; they are freely distributed as observed in stages II, III and IV and or, they form complexes with other elements. The proteins are also deposited in all stages of the oocytes, however, in lower concentration in the stage IV. There is carbohydrate deposition from oocytes in the stage II as well as in oocytes in stages III and IV. The present work has demonstrated that the oocyte yolk of *A. triste* has a glycolipoproteic nature and the elements are deposited in the following sequence: firstly the lipids and proteins, and finally the carbohydrates.

Key words: Tick; Ovary; Vitellogenesis; Cytochemistry; Lipids; Proteins; Carbohydrates.

1. Introduction

Ticks are arthropods of great importance as they can transmit several microrganisms such as viruses, ritckettsias, spirochetides, and agents of scabies and other dermatoses to the human-being and other animals (Rey, 2001). Furthermore, they can jeopardize the cattle-farm-raising business.

The reproductive system of tick females is constituted by an ovary, an uterus, and a vagina. According to Till (1961), in *Rhipicephalus appendiculatus*, Sonenshine, (1994), in *Dermatocentor andersoni* and *D. variabilis*, Denardi et al. (2004), in *Amblyomma cajennense*, Oliveira et al. (2005), in *R. sanguineus,* Saito et al. (2005), in *Boophilus microplus* and Oliveira et al. (*in press*), in *A. triste*, the ovary is a unique, tubular, continuous structure with the shape of a horseshoe that is connected to a genital orifice through one or more oviducts.

The ovaries containing oocytes are involved in the synthesis of yolk. Rosell & Coons (1992), studying *D. variabilis* engorged females, affirmed that the union with the host is the sign that evidences the beginning of this process.

Denardi et al. (2004), studying *A. cajennense* were the first ones to mention the panoistic characteristic of the ovary in ticks. Posteriorly, Oliveira et al. (2005), in *R. sanguineus*, Saito et al. (2005), in *B. microplus* and Oliveira et al. (*in press*), in *A. triste*, obtained the same results. In the panoistic ovaries, all germ cells correspond to the oogonia, which will originate the oocytes, not being found nurse or follicular cells.

Histologically, the tick ovary is an organ comprised of a wall constituted by small cubic cells with round or flat nuclei, where the oocytes in different developmental stages are found attached through a cellular pedicel and that delimitates a narrow lumen (Till, 1961; Denardi et al., 2004; Oliveira et al., 2005; Saito et al., 2005). The number of development stages of the oocytes varies among the species.

Histochemical studies were performed by Denardi et al. (2004), in *A. cajennense*, Oliveira et al. (2005), in *R. sanguineus,* Saito et al. (2005), in *B. microplus* and Oliveira et al. (*in press*), in *A. triste*, allowed the classification of

oocytes in different developmental stages as well as the characterization of chemical components present in the yolk and other ovary cells.

The application of ultrastructural cytochemical techniques allows the detection of chemical compounds in the studied material through the treatment with heavy metal salts for the interactions of these with the electrons and allows the formation of image through black contrast, known as electrondense, and white contrast, known as electronlucid. The quantity of salts impregnated in several cellular constituents is directly proportional to the contrast for the visualization of proteins, carbohydrates, lipids, nucleic acids, ions (or inorganic molecules), and enzymes (Carvalho, 2001).

Studies with *A. triste* ticks are extremely important, once this species is responsible for the transmission of *Rickettsia conorii* (Semtner and Hair, 1973). Furthermore, there is no study characterizing the cytochemical constitution of the yolk of tick. Thus, this work aims to contribute with the first data regarding the deposition sequence of several elements that comprises the yolk of ticks as well as the origin of each one of them – lipids, proteins, and carbohydrates – data that might help in the future control of this parasite.

2. Material and Methods

Semi-engorged females of *Amblyomma triste* ticks used in this study come from the colony submitted to controlled conditions (28º C, 80% humidity, and 12h photoperiod) at the Department of Animal Pathology, Veterinary College, UNESP – Jaboticabal, SP, Brazil. Equipment from the Electron Microscopy Laboratories of the Biology Department at the Biosciences Institute, UNESP – Rio Claro, SP, Brazil, were utilized throughout the study.

2.1. Ultrastructural cytochemistry for the detection of carboydrates (Thiery, apud Haddad et al., 1998)

The ovaries were prepared following the routine for transmission electron microscopy. Once sectioned using an ultramicrotome, the ultrathin sections of

the material were placed on gold meshes, letting the sections float on 1% periodic acid for 15 to 20 minutes. This treatment was followed by successive baths in acetic acid at the following concentrations: 10%, 5%, and 2%, for 10 minutes each. After three 10-minutes washes in distilled water, the meshes with the material were transferred to a solution of 1% silver proteinate for 30 minutes at room temperature and in the dark. Finally, the material was washed once in distilled water and observed through the transmission electron microscope, PHILLIPS TEM 100, without contrasting the material.

2.2. Ultrastructural cytochemistry for the detection of lipids (Angermuller; De Fahimi, apud Haddad et al., 1998)

The ovaries were fixed in 2,5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.2, for 2 hours. The material was then washed once in the same buffer and once in 0.1 M imidazole buffer, pH 7.5, for 10 minutes each. The material was post-fixed in 2% osmium tetroxide in 0.1 M imidazole buffer, pH 7.5. Afterwards, the material underwent two more washes in buffer, followed by acetone dehydration, and then included in Epon resin, according to standard procedures.

The ultrathin sections obtained were observed through the transmission electron microscope (PHILLIPS TEM 100), without undergoing the contrast process.

2.3. Ultrastructural cytochemistry for the detection of basic proteins (Macrae; Meetz, apud Haddad et al., 1998)

The ovaries were fixed in 2.5% glutaraldehyde, washed in distilled water, incubated in ammonium silver solution (10% silver nitrate added to a concentrated solution of ammonium hydroxide) for 5 minutes at room temperature. Next, the material was washed in distilled water and incubated in 3% formaldehyde for 5 minutes, in which the material acquired a brown coloration. Immediately afterwards, the material was washed in distilled water,

post-fixed according to routine procedures and observed through the transmission electron microscope, PHILLIPS TEM 100.

3. Results

For better understanding and comparison, the results were placed in the table I.

Table 1. Ultrastructural cytochemistry detection of carbohydrates, lipids and proteins in the Amblyomma triste ticks ovaries.

Ovary Structures	Carbohydrates	Lipids	Proteins
Wall	- positive; - disperse granules (Figs.1B; 1C); - aggregated granules (Fig. 1C); - large amounts of electrondense material (Fig. 1A).	- negative (Fig. 2A)	- positive; - large amounts of electrondense granules (Fig. 3A); - fine and disperse granulation (Fig. 3A).
Pedicel	- positive; - *granules concentration* (Fig.1E); - fine and disperse granulation (Figs.1D; 1E); - electrondense granules (Fig.1D).	- positive; - few granules, strongly electrondense, usually close to the interdigitations (Figs. 2B; 2C).	- positive; - large amounts of electrondense granules (Figs. 3B; 3C); - fine and disperse granulation (Fig. 3D).
Pedicel/Oocyte Interface	- positive; - electrondense granules in the cytoplasm close to the oocyte plasmic membrane (Fig. 1F); - large amounts of electrondense material among the microvilli (Fig. 1F).	- positive; - strongly electrondense granules close to the oocyte plasmic membrane (Fig. 2B).	- positive; - large amounts of electrondense granules among the microvilli (Figs. 3C; 3D); - fine and disperse granulation among the microvilli (Fig. 3D).

Ovary Structures		Carbohydrates	Lipids	Proteins
OOCYTE I	**Plasmic membrane/basal lamina interface**	- negative	- positive; - small granules strongly electrondense (Fig. 2E).	- positive; - large amounts of electrondense granules (Fig. 3F); - fine and disperse granulation (Fig. 3F).
	Periphery cytoplasm	- negative (Fig. 1G)	- positive; - small granules strongly electrondense (Fig. 2E).	- positive; - fine and disperse granulation (Fig. 3F); - large amounts of electrondense granules at the pedicel cells/oocyte interface (Fig. 3C).
	Central cytoplasm	- negative (Fig. 1H)	- positive; - small granules strongly electrondense (Fig. 2F); - positive mitochondria (Fig. 2F).	- positive; - fine and disperse granulation (Fig. 3G); - large amounts of electrondense granules (Fig. 3G).
	Cytoplasm close to the germinal vesicle	- negative	- positive; - small granules strongly electrondense (Fig. 2G); - positive mitochondria (Fig. 2G).	- positive; - large amounts of electrondense granules in the cytoplasm and inside germinal vesicle (Fig. 3E).

Ovary Structures		Carbohydrates	Lipids	Proteins
OOCYTE II	**Plasmic membrane/basal lamina interface**	- positive; - large amounts of electrondense material (Fig. 1I).	- positive; - small granules strongly electrondense (Fig. 2H).	- positive; - large amounts of electrondense granules (Figs. 3H; 3I); - fine and disperse granulation (Fig.3I).
	Periphery cytoplasm	- positive; - fine and disperse granulation (Fig. 1J); - granule associated to the mitochondria (Fig. 1J).	- positive; - larger granules in large amounts that in the stage I (Fig. 2H); - strongly electrondense granules (Fig. 2H).	- positive; - fine and disperse granulation (Fig. 3H); - large amounts of electrondense granules at the pedicel cells/oocyte interface (Fig. 3C).
	Central cytoplasm	- positive; - rough granulation (Fig. 1K); - rough granulation associated to the mitochondria (Fig. 1K).	- positive; - larger granules in large amounts that in the stage I (Fig. 2I); - strongly electrondense granules (Fig. 2I); - granules with electronlucid center (Fig. 2I); - lipid complex (Fig. 2I).	- positive; - fine and disperse granulation (Fig. 3J); - large amounts of electrondense granules (Fig. 3J).
	Cytoplasm close to the germinal vesicle	- positive; - rough granulation (Fig. 1K); - rough granulation associated to the mitochondria (Fig. 1K).	- positive; - larger granules in large amounts that in the stage I (Fig. 2I); - strongly electrondense granules (Fig. 2I); - granules with electronlucid center (Fig. 2I); - lipid complex (Fig. 2I).	- positive; - fine and disperse granulation (Fig. 3J); - large amounts of electrondense granules (Fig. 3J).

Ovary Structures		Carbohydrates	Lipids	Proteins
OOCYTE III	**Plasmic membrane/ basal lamina interface**	- positive; - fine and disperse granulation (Fig. 1L).	- positive; - small granules strongly electrondense (Fig. 2K).	- positive; - large amounts of granules that in the stages I and II (Fig. 3K); - large amounts of electrondense granules (Figs. 3K; 3L); - fine and disperse granulation; (Figs. 3K; 3L).
	Chorium	- positive; - large amounts of electrondense material (Fig. 1L).	- negative (Fig. 2L)	- negative (Fig. 3L)
	Periphery cytoplasm	- positive; - fine and disperse granulation (Fig. 1M); - granule associated to the mitochondria (Fig. 1M).	- positive; - larger granules and in large amounts that in the stage II (Fig. 2J); - strongly electrondense granules (Figs. 2J; 2N); - granules with electronlucid center (Fig. 2O); - lipid complex (Fig. 2P); - positive mitochondria (Fig. 2Q); - posivite vacuoles (Fig. 2M).	- positive; - large amounts of granules that in the stages I and II (Fig. 3K); - fine and disperse granulation (Fig. 3K); - large amounts of electrondense granules at the pedicel cells/oocyte interface (Fig. 3C).
	Central cytoplasm	- positive; - rough granule (Fig. 1N); - granule associated to the mitochondria (Fig. 1N).	- positive; - larger granules and in large amounts that in the stage II (Fig. 2J); - strongly electrondense granules (Fig. 2N); - granules with electronlucid center (Fig. 2O); - lipid complex (Fig. 2P); - positive mitochondria (Fig. 2Q).	- positive; - large amounts of granules that in the stages I and II (Fig. 3M); - large amounts of electrondense granules (Fig. 3M); - fine and disperse granulation (Fig. 3M).
	Cytoplasm close to the germinal vesicle	- positive; - electrondense nuage (Fig. 1O).	- positive; - larger granules and in large amounts that in the stage II (Fig. 2J); - strongly electrondense granules (Fig.2 N); - granules with electronlucid center (Fig. 2O); - lipid complex (Fig.2 P); - positive mitochondria (Fig. 2Q).	- positive; - large amounts of granules that in the stages I and II (Fig. 3N); - large amounts of electrondense granules (Fig. 3N); - fine and disperse granulation (Fig. 3N).

Ovary Structures		Carbohydrates	Lipids	Proteins
OOCYTE IV	**Plasmic membrane/ basal lamina interface**	- positive; - fine and disperse granulation (Fig. 1Q).	- positive; - small granules strongly electrondense (Fig. 2R).	- positive; - large amounts of electrondense granules (Figs. 3O; 3P); - fine and disperse granulation (Fig. 3P).
	Chorium	- positive; - large amounts of electrondense material (Figs. 1L;1P;1Q).	- negative (Fig. 2S)	- negative (Fig. 3P)
	Periphery cytoplasm	- positive; - fine and disperse granulation (Figs. 1P; 1R; 1S; 1T).	- positive; - large amounts granules that in the stages I, II and III (Fig. 2T); - strongly electrondense granules (Fig. 2T); - lipid complex (Fig. 2T).	- positive; - fine and disperse granulation in less concentration than in the stages I, II and III (Fig. 3Q).
	Central cytoplasm	- positive; - rough granulation (Fig. 1U); - fine and disperse granulation (Figs. 1R; 1S; 1U - granules associated to the mitochondria (Fig. 1U).	- positive; - large amounts granules that in the stages I, II and III (Fig. 2U); - strongly electrondense granules (Fig. 2U); - lipid complex (Fig. 2U).	- positive; - fine and disperse granulation in less concentration than in the stages I, II and III (Fig. 3Q).
	Cytoplasm close to the germinal vesicle	- positive; - electrondense nuage (Fig. 1V).	- positive; - large amounts granules that in the stages I, II and III (Fig. 2U); - strongly electrondense granules (Fig. 2U); - lipid complex (Fig. 2U).	- positive; - fine and disperse granulation in less concentration than in the stages I, II and III (Fig. 3R).

4. Discussion

In the Arthropods, ovaries are classified as meroistic or panoistic and they are comprised by unities denominated ovarioles. The meroistic ovaries are subdivided in polytrophic and telotrophic ovaries; the meroistic ovary are characterized by the presence of nurse cells or trophocytes (Chapman, 1998). Concerning the panoistic ones, all the present germ cells correspond to the oogonia, which will originate the oocytes and there are no presence of nurse and follicular cells.

Ticks present panoistic ovaries constituted by a tubular, continuous structure with a horseshoe shape. Denardi et al. (2004) studying *Amblyomma cajennense* were the first authors to mention this type of ovary in ticks. Posteriorly, Oliveira et al. (*in press*) also classified the ovary of *A. triste* as being of the panoistic type.

With regard to the origin of the yolk in the Arthropod oocytes, several works suggest the possibilities following: through transportation of material from the nurse chamber to the oocyte (when it is present); b) from the follicular epithelium to the oocyte (when it is present); c) throughout the synthesis of the yolk (endogenous); and d) from an external source out of the ovary , mainly the intestine or fat body. However, because of the panoistic type of the ovary in ticks, therefore not accompanied by nurse and follicular cells, it is discarded the hypothesis of transportation of material from these cells to the oocyte. According to Balashov (1983), in ticks, the material from the hemolymph that is captured by the oocytes could be originated in the intestine cells and according to Sonenshine (1994), it could be originated in the cells of the fat body. The literature also reports with regard to ticks that there is transportation of substances from the pedicel cells to the oocytes as well as the vitellogenesis from the oocyte, that is, endogenous production.

The oocytes of ticks are attached to the ovary wall through the pedicel, a structure resulting of the proliferation of epithelial cells from the wall (Till, 1961). In the stage IV, the oocytes detach from the pedicel and they are released in the ovarian lumen (Oliveira et al, *in press*). According to Denardi et al. (2004), in *A. cajennense*, and Oliveira et al. (2005), in *R. sanguineus*, the development of

oocytes is not synchronized and Denardi et al. (2004) suggested that the presence of oocytes in different stages of maturation could point the occurrence of vitellogenesis in different periods, therefore allowing these cells to be fertilized in different periods as well. On the other hand, Oliveira et al. (*in press*) suggested that the development and the oocyte release in *A. triste* happened synchronically once in all stages the oocytes are attached to the pedicel and they present few cytoplasmic variations.

According to Ramamurty (1968), in the study with insects, and other authors with arthropods (Denardi et al., 2004), the yolk is constituted by lipids, which are deposited firstly, then the proteins, and the polysaccharides, which are the last one to be deposited and presented to be free or chemically bounded to other elements. In contraposition, Balashov (1983) registered that the yolk in ticks would be constituted only by proteins and lipids. Denardi et al. (2004) and Oliveira et al. (2005), confirmed in ticks the data obtained by Ramamurty (1968), through the histochemical analysis in ovaries of *A. cajennense* and *R. sanguineus*, respectively, which revealed that the oocytes in stage I present the high lipid content, few protein and almost no polysaccharide; oocytes in stages II present significant quantity of lipids and proteins, nevertheless they present few polysaccharides and oocytes in stages III, IV and V present those three elements in high quantities.

The ultrastructural cytochemical studies have showed that in *A. triste* the carbohydrate are present in cells of the ovarian wall, suggesting that this component are produced and/or captured from the hemolymph by such cells, which would use or transport the carbohydrate, posteriorly, to the oocyte through the pedicel. In the pedicel, the carbohydrates were observed in the cells that attach oocytes in stages I, II, III or IV. Camargo-Mathias (1993) have suggested that in *Neoponera villosa* ants, the follicular epithelium would have a permissive participation in the polysaccharide deposition in oocytes in stage II, corroborating with the data of Ramamurty (1968), who suggest that carbohydrates such as trealose – the sugar of the insect blood – would be transported to the peripheral cytoplasm of the oocyte by diffusion through the follicular epithelium. Camargo-Mathias et al. (1998) also observed that in the

diplopod *Rhinocricus padbergi,* the ovarian epithelium of oocytes in stage II would participate actively in the polysaccharides synthesis or incorporation for the interior of the oocyte.

In *A. triste*, at the regions of contact pedicel cells and at the pedicel cells/oocytes interface, there were found carbohydrates that suggest the existence of material exchange among these cells and that, specifically, the pedicel cells contribute with the carbohydrate supply to the oocyte, which posteriorly will constitute the yolk, therefore confirming that the pedicel is an active structure in the element supply of the ticks oocytes. It can be inferred that the pedicel cells could produce carbohydrate and/or be the way of capture of this element of the hemolymph, which posteriorly would be passed to the oocytes. However, the cytochemical positivity for carbohydrates did not happen in all the cells of a pedicel; thus, the carbohydrate were evident only in cells that is in direct contact to the oocyte. It can be stated that the pedicel cells work asynchronically and only those cells in contact to the oocytes produce more actively the carbohydrates that will constitute the yolk.

In the oocytes of *A. triste* in stage I, the carbohydrates were not evidenced. This fact points that the synthesis and/or incorporation of this element starts in oocytes in stage II, confirming the results obtained by Oliveira et al. (*in press*) in light microscopy analysis.

Few carbohydrate were evidenced in the oocytes in stage II of *A. triste*. The presence of such element, especially at the interface of the plasmic membrane with basal lamina could point that they would be directly captured from the hemolymph. Furthermore, the mitochondria present in this region would be possible energy sources for the incorporation process via plasmic membrane. Therefore, in *A. triste*, the carbohydrates in oocytes in stage II would have two sources of origin: the production and transportation of this element through the pedicel cells, a process also observed by Oliveira et al. (2005), which occurs in *R. sanguineus* oocytes in stage II; and the capture of the hemolymph through the oocyte plasmic membrane.

Therefore, the results obtained in the present work for the oocytes of *A. triste* in stages I and II confirm the data obtained through histochemical studies

for the localization of carbohydrates, by Denardi et al. (2004) in *A. cajennense* and by Oliveira et al. (2005) in *R. sanguineus*. On the other hand, Saito et al. (2005) verified that in ovaries of *B. microplus*, the oocytes in stages I and II presented PAS positive rough granules of yolk disperse in the entire cytoplasm.

The oocytes of *A. triste* in stage III have presented the largest quantity of carbohydrate in relation to those ones in stage II, which were evidenced in the chorion region pointing the presence of that element in this structure as well as in the cortical cytoplasm and central cytoplasm of the oocyte. The presence of carbohydrates at the plasmic membrane invaginations pointed that, despite the deposition of the chorion to be occuring in oocytes III, the capture process of this element from the hemolymph and/or from pedicel cells is undergoing in the oocytes in this stage. The largest concentration of carbohydrate in the central cytoplasm confirmed the results obtained by Oliveira et al. (*in press*) in histochemical analysis, which suggested that after its capture the carbohydrate would accumulate at the central region. In this stage, the hypothesis of active synthesis of carbohydrate at the central region of the oocytes is not discarded.

Denardi et al. (2004) observed that in oocytes of *A. cajennense* in the stage III, the carbohydrates were slightly marked, and they were evidenced mainly at the nuage region. However, Oliveira et al. (2005) verified that the oocytes of *R. sanguineus* in stage III presented strongly positive yolk granules, being the largest ones located mainly at the periphery of the oocytes and the smallest ones at the central region. Saito et al. (2005) observed oocyte of *B. microplus* in stage III with rough yolk granules and positive to the histochemical test and larger than others in anterior stages. Differently of *R. sanguineus* and *B. microplus*, the oocytes of *A. triste* in stage III have presented the peripheral cytoplasm with positive fine granulation and the central cytoplasm with rough aspect more evident.

With regard to oocytes in stage IV, the presence of carbohydrate at the plasmic membrane invaginations indicated that, despite the chorion to be almost entirely deposited, there is still occurring the apprehension of this component from the hemolymph and/or by pedicel cells. It is not discarded also the hypothesis of endogenous production of carbohydrates. The detection of

rough granulation at the central region of the oocytes was also observed by Denardi et al. (2004), in *A. cajennense*. However, Oliveira et al. (2005), in *R. sanguineus*, and Saito et al. (2005), in *B. microplus*, detected smaller granules at the central region and the largest ones at the peripheral region.

The second element analyzed in the present study were the lipids, which were not evidenced in the cells of the ovarian walls of *A. triste*, indicating their no contribution for the supply of this element in the constitution of the yolk in the oocytes of this species. According to Camargo-Mathias (1993), ultrastructural and histochemical analyses of *N. villosa* ants revealed that cells of the ovarian wall and follicular epithelium of oocytes in stages I and II do not participate significantly in the lipid deposition in the oocyte of these insects. Furthermore, Camargo-Mathias et al. (1998) observed in diplopod *R. padbergi,* that the cells of the follicular epithelium of oocytes in intermediary stage are probably a site of production of lipids, which posteriorly would be transferred to the oocytes.

The pedicel cells of ovaries of *A. triste* contributes with the lipid supply for the yolk constitution since as this component was found at the region of contact pedicel/oocyte as well as at the cytoplasm close to the interdigitations among the pedicel cells, which indicates a possible cell-to-cell lipid transference and finally to the interior of the oocyte.

The presence of stock lipids in *A. triste* demonstrates the beginning of the deposit process in oocytes in stage I, confirming the histochemical data of Oliveira et al. (*in press*), which revealed a homogeneous cytoplasm with moderate positivity in oocytes in stage I. Denardi et al. (2004), Oliveira et al. (2005) and Saito et al. (2005) found a homogeneous cytoplasm, however, strongly positive to the lipids in oocytes in stage I of *A. cajennense*, *R. sanguineus* and *B. micropilus*, respectively. Different results were found also by Camargo-Mathias (1993), regarding the presence of lipids, who verified a heterogeneous positivity in oocytes in stage I of *N. villosa* ants; and by Camargo-Mathias et al. (1998), who verified the complete negativity in oocytes in stage I of the diplopod *R. padbergi*.

Regarding *A. triste*, the presence of lipids at the interface of the oocyte plasmic membrane with basal lamina indicates that be happening the capture of

this element from the hemolymph by the oocyte. The exogenous incorporation was also suggested by Oliveira et al. (2005), in studies with *R. sanguineus*. The fusion of existing granules in the cytoplasm characterizes a homogeneous distribution of them, which can be seen due to the occurrence of endogenous production as well as exogenous incorporation, where smaller granules captured from the hemolymph or produced by the oocyte and by the cells of the pedicel are joined forming larger granules.

The presence of lipids in mitochondria of oocytes in *A. triste* points their involvement with the production of this element and/or the use of it as energy source on its own oxidative processes. Ranade (1933) affirmed that the oocyte mitochondria of *Periplaneta americana* would be transformed in lipid bodies of yolk and Boissan (1970) suggested that the lipid material of oocytes of *Hysterochelifer meridianus* were originated from the break and destruction of mitochondrial crests. The same was suggested by Camargo-Mathias (1993) in oocytes of *N. villosa* ants; by Oliveira et al. (2005), in *R. sanguineus* and by Oliveira et al. (in press), in *A. triste*, since the non-granular endoplasmic reticulum was not found in the oocytes, the principal organelle responsible for the synthesis of lipids; and by Denardi et al. (*in press*) in salivary gland cells of *A. cajennense*.

However, in *A. triste* oocytes in stages II, III and IV, there was also observed lipids forming complexes with other elements as there was noticed the presence of granules with center more electrondense than the periphery and vice-versa. Camargo-Mathias et al. (1998) also verified the absence of exclusively lipid granules of yolk in oocytes of diplopods *R. padbergi*. The granules in *A. triste* that were presented with center more electronlucid and the periphery more electrondense can indicate that this lipid is suffering modifications, characteristic also observed by Camargo-Mathias (1993) in oocytes in stage II of *N. villosa* ants.

The third element cytochemically analyzed in the oocytes of *A. triste* was the protein, which was detected in high quantity in cells of the ovarian wall, mainly at the interface of the plasmic membrane with the basal lamina, suggesting that they produce and/or incorporate this element, which will be,

afterwards, transported to the oocytes and/or used for its metabolism. These results confirm the histochemical data obtained by Oliveira et al. (*in press*) in *A. triste*; such data reports the exhibition of the plasmic membrane and of the cytoplasm middling positive to the Bromophenol Blue stain.

In *A. triste*, the proteins were also evidenced in the pedicel cells; their distribution has indicated that they produce and/or incorporate proteins from the hemolymph, which will be transported posteriorly to the oocytes as well as be used for themselves. The presence of such proteins at the pedicel cells/oocyte interface confirms the transference of this material to the oocyte and it also the participation of the pedicel as a structure that supply the elements of the yolk for oocytes of this species of tick. In contraposition, Oliveira et al. (2005) observed that pedicel cells in *R. sanguineus* had slight positivity for protein elements.

In the present work, the cytochemical test confirmed that in *A triste* the distribution of the proteins was the same one in oocytes in stages I, II and III, that is, larger concentration in the central cytoplasm as well as close to the germinal vesicle. This reinforces that proteins from the exogenous incorporation through the oocyte surface and through the contact of pedicel/oocyte are getting concentrated in these regions, not discarding the hypothesis of the endogenous synthesis of proteins. Nevertheless, this concentration increased significantly in the oocytes in stages II and III. Among the proteins that were evidenced in the germinal vesicle it is possible to include the hystones. The distribution and the concentration of basic proteins in the oocytes in stage IV was different when compared to the three previous ones. In this stage, the accumulation of the electrondense protein granules in the cytoplasm and inside germinal vesicle rarely occurred, pointing probably the formation of complexes by this protein with other elements. The fine protein granules disperse in the entire oocyte also were found in lower concentration.

Differently from observed in the cytochemical study in *A. triste*, Oliveira et al. (2005) verified in *R. sanguineus* that the oocytes in stage I had slightly positivity for protein elements. In oocytes in stage II was detected a fine granulation uniformly distributed in all the cytoplasm. Oocytes in stage III presented small protein granules close to the germinal vesicle, and larger

granules bellow the plasmic membrane; Denardi et al. (2004) verified in *A. cajennense* that in all stages the oocytes had strongly positive reaction.

Saito et al. (2005) performing histochemical analyses of proteins in the ovaries of *B. microplus* obtained also different results of the ones here presented for *A. triste*, once they observed the presence of proteins in the plasmic membrane as well as inside all oocytes. However, the material in the cytoplasm was not uniformly distributed: in stages I and II, the proteins are presented homogenously disperse; in the stage III with fine granulation; in the stage IV, the granules were larger; and in the stage V and VI, the proteins presented as large granules of yolk.

According to Camargo-Mathias et al. (1998), the oocytes in stages I, II and III of diplopods *R. padbergi* reacted positively to the test for the detection of protein as occurred to the follicular epithelium of oocytes of *N. villosa* ants in stages I and II, suggesting, in this case, that there is exogenous incorporation of proteins. According still to Carmargo-Mathias (1993), the follicular epithelium of oocytes in stage I presented slightly positive to the test of protein detection. In the peripheric cytoplasm of oocytes in stage I, there was observed a stronger reaction than in its central portion. The author suggested that the large quantity of granular endoplasmic reticulum in the peripheral regions of oocytes in this stage would point to an intense synthesis of proteins, therefore, of an endogenous origin. In oocytes in stage II, there were observed a rougher granulation in the periphery, where strongly positive protein spheres of several sizes were found. The follicular epithelium of oocytes in stage II had strongly positive reaction in determined sites; however in others, the reaction was slightly positive, supporting the conclusion that not every cell of the follicular epithelium function synchronically as observed in pedicel cells in *A. triste*. The follicular epithelium of oocytes in stage III presented strongly positive and large spheres of yolk were presented in the cytoplasm of the oocytes. According to the author, the presence of granular endoplasmic reticulum in oocytes in stages II and III would indicate the activation of a site of extra-ovarian protein synthesis from these stages on.

Thus, in tick *A. triste*, the proteins could have double origin: endogenous, through its production by the oocytes as the presence of strong positivity in region of nuage around the germinal vesicle could be indicating the beginning of information exchange among the nucleus and the oocyte cytoplasm, and the other exogenous one, where the pedicel cells and the hemolymph would be acting as suppliers of this element for the composition of the yolk of the oocytes in this species of tick.

5. Acknowledgments

We thank to Miss Monika Iamonte, Mr. Antonio Teruyoshi Yabuki, Mr. Ronaldo Del Vecchio and Cristiane Marcia Mileo for their technical support and FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo) for the financial support (Grants n°. 04/02680-0 and n°. 02/10149-7).

6. Figure of Legends

Figure 1. Transmission Electron Microscopy of *Amblyomma triste* ovarian cells submitted to the cytochemical test for carbohydrate detection.

A, **B and C.** Epithelial cells **(epo)** of the ovarian wall: **n** = nucleus; **nu** = nucleolus; **A.** → = large amounts of electrondense material. **B.** → = disperse carbohydrate granules. **C.** → = disperse carbohydrate granules; → = aggregated granules. **D.** Detail of the pedicel cells **(pc)**: **i** = interdigitations among the pedicel cells; → = electrondense carbohydrate granules; → = fine and disperse granulation. **E.** Detail of the pedicel cells **(pc)**: → = fine and disperse granulation; → = granules concentration. **F.** Detail of the pedicel cells **(pc)**/oocyte **(ov)** interface (positive at the pedicel cells/oocyte interface of oocytes I, II, III or IV): **mv** = microvilli; → = large amounts of electrondense material; → = electrondense carbohydrate granules. **G.** Detail of the oocyte I periphery **(ov I)** showing negativity to the test: **m** = mitochondria. **H.** Detail of the oocyte I **(ov I)** center showing negativity to the test: **m** = mitochondria. **I.** Oocyte II **(ov II)**. Contact region of the plasmic membrane **(pm)** with the basal

lamina **(bl)**: →= large amounts of electrondense material. **J.** Detail of the oocyte II periphery **(ov II)**: **m** = mitochondria; → = fine and disperse granulation; →= carbohydrate granule associated to the mitochondria. **K.** Detail of the central cytoplasm and close to the germinal vesicle of oocyte II **(ov II)**: →= rough granulation associated to the mitochondria **(m)**. **L.** Oocyte III **(ov III)** and IV **(ov IV)** periphery: chorion **(ch)**; basal lamina **(bl)**; →= fine and disperse granulation. **M.** Detail of the oocyte III periphery **(ov III)**: **m**= mitochondria; **sg** = secretion granule ; →= carbohydrate granule associated to the mitochondria; → = fine and disperse granulation. **N.** Details of the oocyte III center **(ov III)**: **m**= mitochondria; →= rough granulation associated to the mitochondria; **O.** Detail of the region close to the germinal vesicle **(gv)** of oocyte III **(ov III)**: **na** = nuage →= electrondense carbohydrate granules. **P.** Detail of the oocyte IV periphery **(ov IV)**: **va** = vacuole; **bl** = basal lamina; **ch** = chorion; **in** = oocyte plasmic membrane invaginations; →= fine granulation; **Q.** Detail of the oocyte IV periphery **(ov IV)**: chorion **(ch)** and plasmic membrane **(pm)**/basal lamina contact **(bl)**: **in** = plasmic membrane invaginations; →= disperse carbohydrate granules. **R, S.** Details of vacuoles **(va)** in the cytoplasm of the oocyte IV **(ov IV)**: →= fine granulation. **T.** Detail of the oocyte IV periphery **(ov IV)**: →= fine and disperse granulation. **U.** Detail of the oocyte IV center **(ov IV)**: **m** = mitochondria; →= fine and disperse granulation; →= rough granule; →= granules associated to the mitochondria. **V.** Detail of the region close to the oocyte IV **(ov IV)** germinal vesicle **(gv)**: **na** = nuage: →= electrondense carbohydrate granules.

Figure 2. Transmission Electron Microscopy of *Amblyomma triste* ovarian cells submitted to the cytochemical test for lipid detection.

A. Epithelial cells **(epo)** of the ovarian wall showing negativity to the test: **pm** = plasmic membrane; **m** = mitochondria; **B.** Pedicel cells **(pc)**/oocyte **(ov)** interface. **i** = interdigitation among the pedicel cells; → = lipid granules at the oocyte/pedicel interface; →= lipid granule at the interdigitation region. **C.** Interdigitation region **(i)** among the pedicel cells **(pc)**: →= lipid granule. **D.** Overview of the oocyte I **(ov I)**: **pm** = plasmic membrane; **gv** = germinal vesicle;

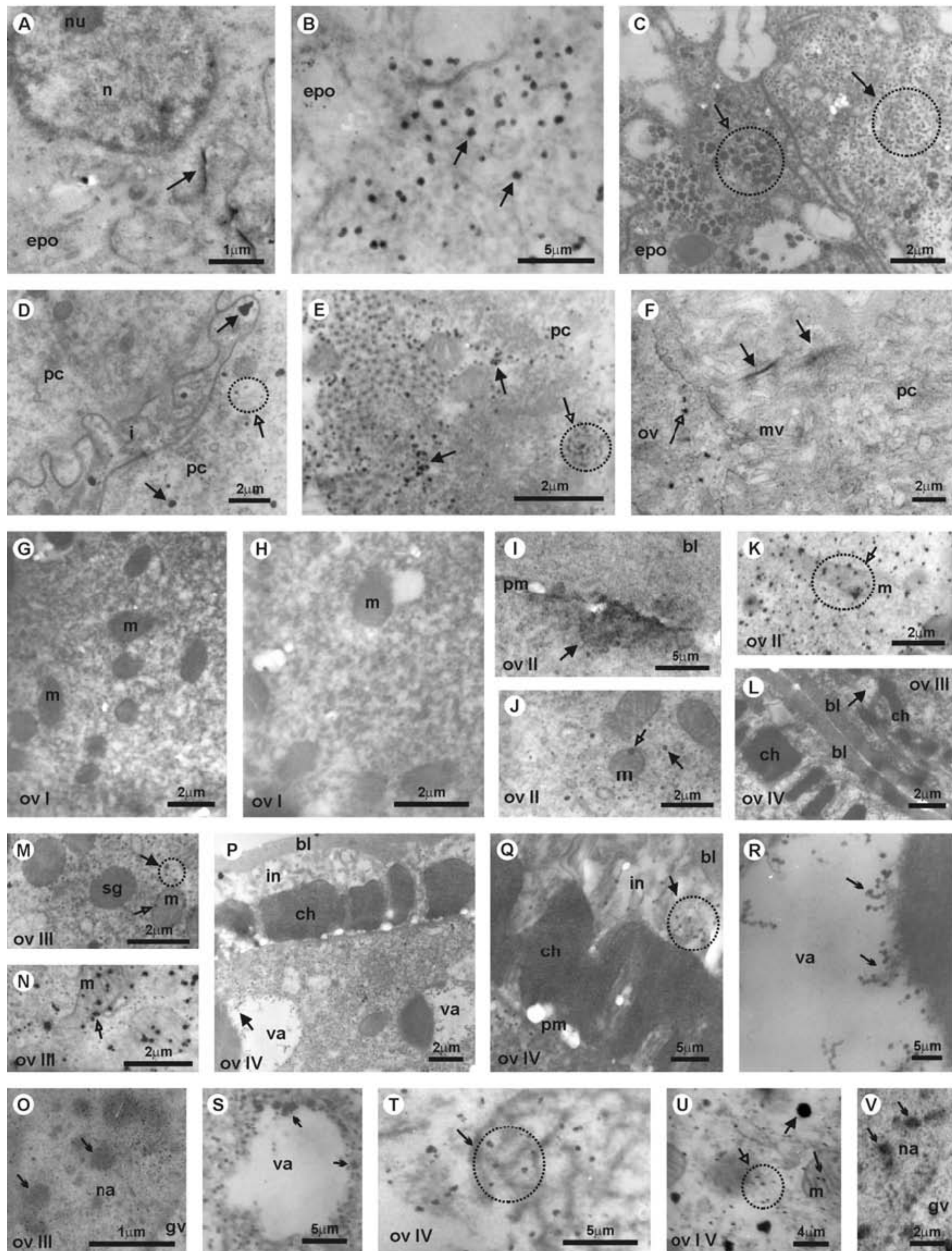
→= lipid granules in the cytoplasm. **E.** Detail of the oocyte I periphery **(ov I)**: **bl** = basal lamina; **in** = oocyte I plasmic membrane invaginations; → = lipid granule at the region of contact among the plasmic membrane with the basal lamina; → = lipid granules fusion in the peripheral cytoplasm. **F.** Detail of the oocyte I **(ov I)** center: **m**= mitochondria; → = lipid granules associated to the mitochondria; → = granule fusion. **G.** Detail of the region close to the germinal vesicle **(gv)** of oocyte I **(ov I)**: **m** = mitochondria; → = lipid granule associated to the mitochondria; → = granules fusion. **H.** Detail of the oocyte II periphery **(ov II)**: **in** = oocyte plasmic membrane invaginations; **m** = mitochondria; → = lipid granule at the plasmic membrane/basal lamina contact;. → = lipid granule. **I.** Oocyte II **(ov II)**: central cytoplasm and close to the germinal vesicle **(gv)**: lipid granules of several sizes in the cytoplasm: **lc** = lipid complex; **sg** = secretion granule; →= granule with electronlucid center; →= strongly electrondense granule. **J.** Overview of the oocyte III **(ov III)**: → lipid granules of several sizes in the cytoplasm. **K.** Oocyte III **(ov III)**: plasmic membrane **(pm)**/basal lamina **(bl)** contact: **in** = plasmic membrane invaginations; → = lipid granules. **L.** Oocyte III **(ov III)** periphery: chorion **(ch)**; **bl** = basal lamina; **in** = oocyte plasmic membrane invaginations. **M.** Oocyte III **(ov III)**: Detail of vacuoles **(va)** distributed mainly at the oocyte periphery: → = lipid granule in the vacuole periphery. **N.** Oocyte III **(ov III)**: Detail of the lipid granules: → = lipid granules in fusion process; **m** = mitochondria. **O.** Oocyte III **(ov III)**: Detail of lipid granules found in the cytoplasm: → = strongly electrondense lipid granule; → = lipid granule with electronlucid center; **va** = vacuole. **P.** Oocyte III **(ov III)**: Detail of lipid complex **(lc)** in the cytoplasm: **sg** = secretion granule . **Q.** Oocyte III **(ov III)**: Detail of disorganized mitochondria **(m)**: → = lipid granule associated to the mitochondria; → = lipid granules associated to the disorganized mitochondrial membranes. **R.** Detail of the oocyte IV periphery **(ov IV)**: **in** = oocyte plasmic membrane invaginations; → = lipid granules. **S, T.** Details of the oocyte IV periphery **(ov IV)**: **ch** = chorion; **lc** = lipid complex; → = lipid fusion of strongly electrondense granules; **sg** = secretion granule . **U.** Detail of central cytoplasm and close to the oocyte IV **(ov IV)** germinal vesicle: **lc** = lipid complex; → = lipid granule fusion; **sg** = secretion granule .

Figure 3. Transmission Electron Microscopy of *Amblyomma triste* ovarian cells submitted to the cytochemical test for protein detection.

A. Epithelial cells **(epo)** of the ovarian wall: **bl** = basal lamina; →= large amount of electrondense protein granules; → = fine and disperse granulation. **B.** Detail of the pedicel cells **(pc)**: **bl** = basal lamina; **n** = nucleus; **nu** = nucleolus; → = large amounts of electrondense protein granules. **C.** Detail of the pedicel **(pc)**/oocytes **(ov)** contact (positive in oocytes I, II, III and IV): →= large amounts of electrondense protein granule in the cytoplasm close to the pedicel cells/oocyte contact. **D.** Detail of the pedicel cells **(pc)**/oocyte **(ov)** contact: →= large amounts of electrondense protein granules at the pedicel cells/oocyte interface; → = fine and disperse granulation. **E.** Oocyte I **(ov I)** in overview: **gv** = germinal vesicle; →= protein granules; →= protein granule inside germinal vesicle. **F.** Detail of the oocyte I periphery **(ov I)**: **bl** = basal lamina; **in** = oocyte I plasmic membrane invaginations; **m** = mitochondria; →= large amounts of electrondense protein granules at the region of contact among the plasmic membrane and basal lamina; → = fine and disperse granulation at the oocyte periphery; →= fine and disperse granulation at the plasmic membrane/basal lamina interface. **G.** Detail of the oocyte I **(ov I)** center: **m** = mitochondria; →= large amount of electrondense protein granules; → = fine and disperse granulation; **H, I.** Details of the oocyte II periphery **(ov II)**: **bl** = basal lamina; **in** = oocyte plasmic membrane invaginations; **m** = mitochondria; →= large amounts of electrondense protein granules; → = fine and disperse granulation; → fine granulation. **J.** Detail of the central cytoplasm and close to the germinal vesicle of oocyte II **(ov II)**: →= large amounts of electrondense protein granules; → = fine and disperse granulation. **K, L.** Details of the oocyte III **(ov III)** periphery: **bl** = basal lamina; **ch** = chorion; →= large amounts of electrondense protein granules; →= fine and disperse granulation. **M.** Detail of the center of the oocyte III **(ov III)**: **va** = vacuole; **sg** = secretion granule; → = large amounts of electrondense protein granules; → = fine and disperse granulation. **N.** Detail of the region close to the germinal vesicle **(gv)** of oocyte III **(ov III)**: → = large amounts of electrondense protein granules; → = fine and disperse granulation.

O. Oocyte IV **(ov IV)** in overview: →= large amounts of electrondense protein granules at the plasmic membrane/basal lamina contact. **P.** Detail of the oocyte IV **(ov IV)** periphery: **bl** = basal lamina; **ch** = chorion; →= large amounts of electrondense protein granules; → fine granulation. **Q.** Detail of the peripheral and central oocyte IV **(ov IV)** cytoplasm: **sg** = secretion granule ; →= fine and disperse granulation. **R.** Detail of the region close to the germinal vesicle **(gv)** of oocyte IV **(ov IV)**: →= large amounts of electrondense protein granules.

FIGURE 1



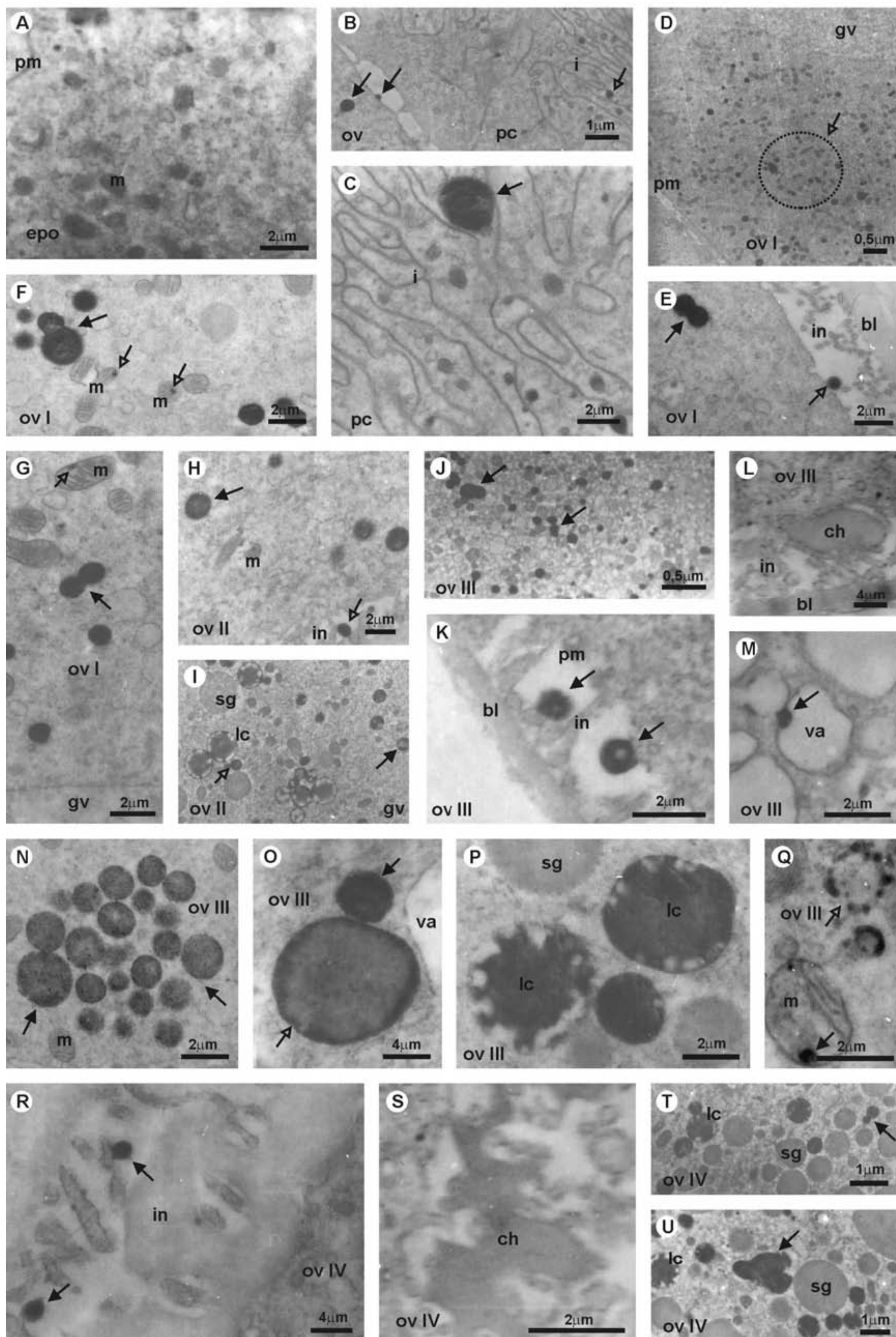
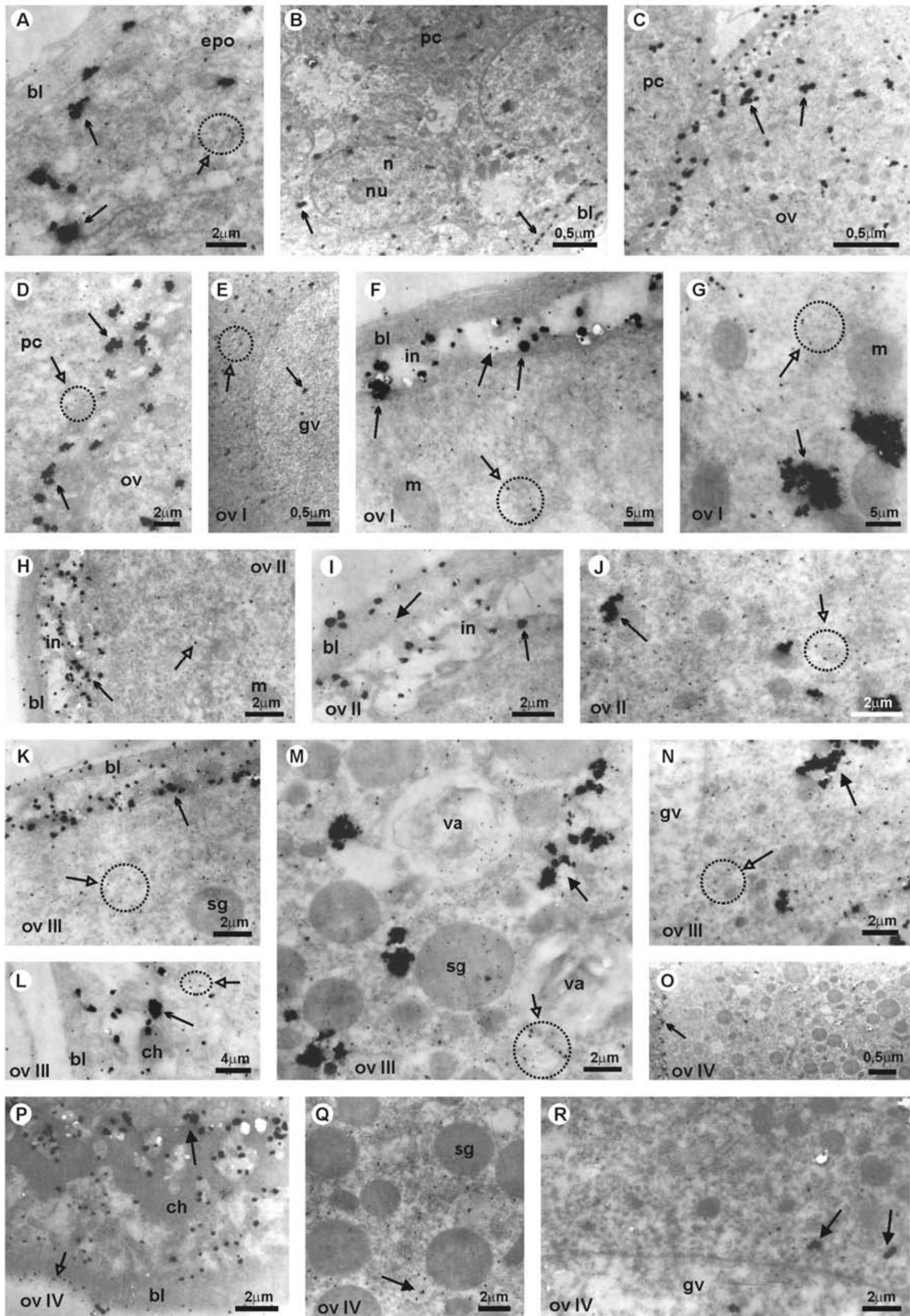


FIGURE 2
A
pm
m
epo
2μm
B
i
ov
pc
1μm
C
i
pc
2μm
D
gv
pm
ov I
0,5μm
E
in
bl
ov I
2μm
F
m
m
ov I
2μm
G
m
ov I
gv
2μm
H
m
ov II
in
2μm
I
sg
lc
ov II
gv
J
ov III
0,5μm
K
pm
bl
in
ov III
2μm
L
ov III
ch
in
bl
4μm
M
va
ov III
2μm
N
ov III
m
2μm
O
ov III
va
4μm
P
sg
lc
lc
ov III
2μm
Q
ov III
m
2μm
R
in
ov IV
4μm
S
ch
ov IV
2μm
T
lc
sg
ov IV
1μm
U
lc
sg
ov IV
1μm

FIGURE 3



7. References

Balashov, Y.S., 1983. The female reproductive system. In: Balashov, Y.S. (Ed.), An Atlas of Ixodid Tick Ultrastructure. Entomological Society of America, Russian, pp. 98-128.

Boissan, L., 1970. Gametogenese au cours du development pos embryonaire et biologie de la reproduction chez *Hysterochelifer meridianus* (L. Lock) Arachnidae: Pseudoscorpion. Doctorat These. Facultat de Sc. De Montpelier, France.

Camargo-Mathias, M.I., 1993. Histoquímica e ultra-estrutura dos ovários de operárias e rainhas de formigas *Neoponera villosa* (Hymenoptera: Ponerinae). Tese (Doutorado em Zoologia) – Instituto de Biociências, Universidade Estadual Paulista, Rio Claro, 156 pp.

Camargo-Mathias, M.I., Fontanetti, C.S., Micó-Balaguer, E., 1998. Histochemical studies of *Rhinocricus padbergi* Verhoeff ovaries (Diplopoda, Spirobolida, Rhinocricidae). Cytobios 94, 169-184.

Carvalho, H.F.; Recco-Pimentel, S.M., 2001. A Célula 2001. Manole Ltda, Barueri, 287 pp.

Chapman, F.R., 1998. The Insects: Structure and Function. Melbourne, 770 pp.

Denardi, S.E., Bechara, G.H., Camargo-Mathias, M.I., *in press*. Salivary gland cells of *Amblyomma cajennense* (Acari: Ixodidae) partially engorged females ticks. Lipids production by mitochondria. Experimental Parasitology.

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 125, 379-395.

El Said, A., 1992. A contribution to the anatomy and histology of the female reproductive system of *Amblyomma cajennense* (Acarina: Ixodidae). J. Egypt. Soc. Parasitol. 22, 391-400.

Haddad, A.; Sesso, A.; Attias, M.; Farina, M.; Meirelles, M.N.; Silveira, M.; Benchimol, M.; Soares, M.J.; Barth, O.M.; Machado, R.D.; Souto-Padrón, T.; Souza, W., 1998. Técnicas Básicas de Microscopia Eletrônica aplicadas às Ciências Biológicas. In: Wanderley de Souza, pp. 66-73.

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

Oliveira, P.R., Camargo-Mathias, M.I., Bechara, G.H., *in press*. Females Ticks of *Amblyomma triste* (Kock, 1844) (Acari: Ixodidae). Ovaries description and vitellogenesis process. Experimental Parasitology.

Ramamurty, P.S., 1968. Origin and distribution of glycogen during vitellogenesis of the scorpion fly *Panorpa communis*. J. Insect. Physiol 14, 1325-1330.

Ranade, V., 1933. On the cytoplasmic inclusions in the oogenesis of *Periplaneta americana*. Linn. Allahad. Univ. Jlud Sci. 9, 85-121.

Rey, L., 2001. Parasitos e Doenças Parasitárias do Homem nas Américas e na África. Guanabara Koogan, Rio de Janeiro, pp. 752-759.

Rosell, R., Coons, L.B., 1992. The role of the fat-body, midgut and ovary in vitellogenin production and vitellogenesis in the female tick, *Dermacentor variabilis*. International Journal for Parasitology 22, 341-349.

Saito, K.C., Bechara, G.H., Nunes, E.T., Oliveira, P.R., Denardi, S.E., 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 129, 299-311.

Semtner, P.J., Hair J.A., 1973. Distribution, seasonal abundance, and hosts of the Gulf Coast tick in Oklahoma. Annais Entomological Soc. Amer. 66, 1264-1268.

Sonenshine, D.E., 1994. Biology of ticks. Oxford University Press, New York, pp. 280-303.

Till, W.M., 1961. A contribuition to the anatomy and histology of the brown ear tick *Rhipicephalus appendiculatus Neumann*. Mem. Entomol. Soc. Southern Africa 6, 65-124.

DISCUSSÃO GERAL

O presente estudo realizado com ovários de carrapatos adultos de *Amblyomma triste* revelou que este é único, tubular, contínuo e em forma de ferradura. Estes dados corroboram os obtidos por Till (1961) em *Rhipicephalus appendiculatus*, Sonenshine (1991) em *Dermacentor andersoni* e *D. variabilis,* Denardi et al. (2004) em *A. cajennense*, Oliveira et al. (2005) em *R. sanguineus* e Saito et al. (2005), em *Boophilus microplus.*

O ovário do carrapato *A. triste* é do tipo panoístico, onde todas as células germinativas presentes correspondem a ovogônias, ou futuros ovócitos, não possuindo portanto, nem células foliculares nem nutridoras especializadas. Essa classificação é a mesma descrita por Denardi et al. (2004)*,* Oliveira et al. (2005) e Saito et al. (2005), nos carrapatos das espécies *A. cajennense*, *R. sanguineus* e *B. microplus,* respectivamente.

Segundo Denardi et al. (2004), estudando os ovários de *A. cajennense* e Oliveira et al. (2005), estudando os de *R. sanguineus*, o desenvolvimento dos ovócitos nestas duas espécies de carrapatos é assincrônico, uma vez que a ovogênese ocorre no sentido distal-proximal ao longo do ovário ficando os ovócitos menos desenvolvidos na região distal, enquanto que os mais

desenvolvidos, na região proximal. No caso de *A. triste*, o desenvolvimento dos ovócitos localizados tanto na região distal quanto na proximal ocorre sincronicamente e os mesmos independentemente do seu estágio possuem poucas variações tanto na forma do ovócito quanto na constituição do seu citoplasma.

Denardi et al. (2004) ainda nesse mesmo estudo sugeriram que a presença de ovócitos em diferentes estágios de maturação em *A. cajennense* poderia estar indicando que a vitelogênese nesta espécie ocorreria em tempos diferentes, capacitando essas células a serem fecundadas também em tempos diferentes. Todavia, em *A. triste* a maturação e conseqüente liberação dos ovócitos para dentro do lúmen do ovário parece ocorrer simultaneamente.

Nos carrapatos em geral, bem como em *A. triste,* os ovócitos nos diferentes estágios de desenvolvimento encontram-se presos à parede do ovário por meio de um pedicelo, estrutura celular resultante da proliferação das células epiteliais desta parede (TILL, 1961). Estes dados corroboram os obtidos por Till (1961) em *R. appendiculatus,* Balashov (1983) em *Hyalomma asiaticum,* Denardi et al. (2004) em *A. cajennense*, Oliveira et al. (2005) em *R. sanguineus* e Saito et al. (2005), em *B. microplus.*

Outros estudos realizados a partir de secções histológicas de ovários de carrapatos por Denardi et al. (2004), mostraram a ocorrência de cinco diferentes estágios de desenvolvimento para os ovócitos da espécie *A. cajennense*. Oliveira et al. (2005) em *R. sanguineus,* relataram a presença de cinco estágios de desenvolvimento além de alguns ovócitos em processo de degeneração, enquanto Saito et al. (2005), de seis estágios de desenvolvimento para ovócitos de *B. microplus.* O presente estudo encontrou a presença de apenas quatro estágios de desenvolvimento para os ovócitos de *A. triste* e ainda revelou pequenas diferenças entre os diferentes estágios dessas células, principalmente no que diz respeito à constituição e à seqüência da deposição dos elementos que compõem o vitelo nos mesmos. Esses dados permitiram classificá-los em estágios que variaram de I a IV, baseada na proposta de Denardi et al. (2004) para os ovócitos de *A. cajennense,* cujos autores consideraram as seguintes características: aspecto citoplasmático,

localização da vesícula germinal, presença, quantidade e constituição dos grânulos de vitelo e presença de cório.

Dessa forma, os **ovócitos I** de *A. triste* apresentaram morfologia arredondada e citoplasma homogêneo, positivo para lipídios e os **ovócitos II**, elípticos e com citoplasma apresentando poucos e pequenos grânulos protéicos, lipídicos e polissacarídicos. Os resultados aqui obtidos para ovócitos I e II de *A. triste* corroboram dados registrados para ovócitos I e II de *A. cajennense* (DENARDI et al., 2004), de *R. sanguineus* (OLIVEIRA et al., 2005) e de *B. microplus* (SAITO et al., 2005).

Os **ovócitos III** de *A. triste* apresentaram forma arredondada e citoplasma com grânulos de vitelo principalmente protéicos e polissacarídicos na região central, a qual foi denominada de **Ce** e, lipídicos maiores formando complexos com outros elementos na região periférica da célula, esta denominada de **Per**. Oliveira et al. (2005) encontraram resultados diferentes em *R. sanguineus* onde grandes grânulos de vitelo protéico, lipídico e polissacarídico ocuparam a região periférica do citoplasma de ovócitos neste mesmo estágio. Já Denardi et al. (2004) em *A. cajennense* e Saito et al. (2005) em *B. microplus,* detectaram granulação grosseira de proteínas, polissacarídios e lipídios distribuída homogeneamente por todo o citoplasma dos ovócitos III destas espécies.

Os **ovócitos IV** de *A. triste* apresentaram morfologia arredondada e citoplasma com pequenos grânulos protéicos e polissacarídicos preferencialmente na região central. Na região periférica foram observados grânulos maiores de natureza lipídica formando complexos com outros elementos e na região intermediária (**In**), foi marcante a presença de grandes grânulos protéicos. Em contraposição, Oliveira et. al. (2005) observaram nos ovócitos IV de *R. sanguineus* numerosos grânulos protéicos e polissacarídicos de vários tamanhos bem como lipídicos, que estavam distribuídos por todo o citoplasma e, Denardi et al. (2004) e Saito et al. (2005) encontraram grandes grânulos de vitelo protéico e polissacarídico que ocupavam a região central e grânulos menores lipídicos, a região periférica de ovócitos IV de *A. cajennense* e de *B. microplus,* respectivamente.

Estudos específicos tratando da reprodução nos Arthropoda em geral, têm demonstrado, principalmente aqueles realizados com insetos, que o vitelo dos ovócitos é constituído por lipídios, proteínas e polissacarídios, que são depositados segundo uma seqüência: primeiramente os lipídios, depois as proteínas e por último os polissacarídios, os quais aparecem livres ou quimicamente ligados a outros elementos. Nos carrapatos esses mesmos elementos foram detectados nos ovócitos, porém Balashov (1983) registrou que o vitelo nas fêmeas deste grupo seria constituído apenas de proteínas e lipídios. O presente estudo, no entanto vem demonstrar que em *A. triste* são encontrados os três elementos e confirma a seqüência de deposição dos mesmos sugerida recentemente por Denardi et al. (2004) e Oliveira et al. (2005), em *A. cajennense* e *R. sanguineus*: onde primeiramente são depositados e/ou sintetizados os lipídios, depois as proteínas e finalmente os polissacarídios.

Estudos abordando os aspectos ultra-estuturais das células que compõem os ovários de carrapatos em geral, são escassos na literatura e os mais recentes são aqueles realizados por Denardi et al. (2004) em *A. cajennense*, Oliveira et al. (2005) em *R. sanguineus* e Saito et al. (2005), em *B. microplus,* já aqueles abordando especificamente as células do pedicelo são praticamente ausentes.

O presente estudo em nível ultra-estrutural demonstrou que em *A. triste,* os ovócitos I e II apresentaram muitas mitocôndrias preferencialmente na periferia e microvilos em sua membrana plasmática, bem como pouco retículo endoplasmático rugoso. A presença das mitocôndrias próximas à membrana plasmática em ovócitos nestes estágios sugere uma preparação dessas células para o período de incorporação exógena do vitelo, no qual estas organelas tornam-se pré-requisito para o transporte ativo de material (BALASHOV, 1972) e, dos microvilos, confirma a ocorrência de alta taxa de incorporação de elementos extra-ovarianos para a composição do vitelo, os quais estariam sendo sintetizados por fontes exógenas e incorporados pelo ovócito via vesículas pinocíticas.

Nos ovócitos III ainda foram encontrados muitos microvilos na membrana plasmática e no citoplasma, muitas mitocôndrias, maior quantidade de grânulos de vitelo e pouco retículo endoplasmático rugoso e liso. A presença de microvilos e a ausência de organelas envolvidas com a síntese permitiram sugerir que, nos ovócitos III, a maior fonte dos elementos do vitelo ainda seja de origem extra-ovariana, porém não exclui a possibilidade de que neste estágio esteja havendo alguma produção endógena desses elementos. Embora tenham sido observadas muitas mitocôndrias nos ovócitos neste estágio, as mesmas apresentaram modificações, tais como a desorganização de suas cristas e de suas membranas além da presença de lipídios no seu interior. Ranade (1933) afirmou que as mitocôndrias dos ovócitos de *Periplaneta americana* se transformariam em corpos lipídicos de vitelo e Boissan (1970) sugeriu que o material lipídico dos ovócitos de *Hysterochelifer meridianus* seria também oriundo da quebra e destruição das cristas mitocondriais. Dessa forma, os dados obtidos no presente estudo sugerem que, em *A. triste,* os componentes lipídicos de ovócitos III poderiam também ter origem a partir das mitocôndrias.

Nos ovócitos IV de *A. triste* também foram observados microvilos. A permanência destas estruturas sugere que a incorporação de elementos extra-ovarianos é contínua durante todo o processo de vitelogênese e que ocorre sempre em quantidade superior a produção endógena de vitelo. No entanto, a presença de microvilos em ovócitos neste estágio poderia estar também relacionada com a deposição do cório, constituído nesta espécie por proteínas, lipídios e principalmente por polissacarídios (natureza glicoplipoprotéica).

A produção endógena de elementos do vitelo, principalmente protéico, nos ovócitos IV de *A. triste* seria definida pela forte marcação na região da "nuage", na periferia da vesícula germinal, que poderia estar significando a troca de informações entre esta e o citoplasma do ovócito. A passagem de material através dos poros nucleares foi também observada nos ovócitos do carídeo *Atya scabra* por Cruz–Landim (1997).

A análise ultra-estrutural das células do pedicelo de *A. triste* revelou que, quando as mesmas estão fixando os ovócitos I e II, são cilíndricas e

apresentam características de células com atividade de síntese, ou seja, com muito retículo endoplasmático rugoso e mitocôndrias e com um núcleo grande com muitos poros e concentração de eucromatina em seu envoltório. Especificamente, os ovócitos II possuem poucos grânulos vitelínicos e pequenas vesículas eletrondensas próximas aos microvilos da sua membrana plasmática em contato com o pedicelo, mas não apresentam retículo endoplasmático liso e rugoso (organelas responsáveis pela síntese de elementos vitelogênicos). Isso permite sugerir que as células do pedicelo de ovócitos II estariam sintetizando parte dos elementos vitelogênicos que seriam transportados para o interior do ovócito, utilizando a energia das muitas mitocôndrias (transporte ativo), as quais estão concentradas na região próxima ao complexo ovócito II/pedicelo

As células do pedicelo que fixam os ovócitos III de *A. triste* tornam-se pavimentosas com núcleos menores e achatados, reduzida quantidade de organelas e muitos vacúolos de pequeno tamanho, demonstrando que essas células começaram a sofrer alterações morfológicas e funcionais à medida que os ovócitos tornaram-se mais desenvolvidos e que essas modificações apareceriam primeiro naquelas em contato direto com o ovócito III, caracterizando o início de um processo de inatividade de síntese e de degeneração. Essa degeneração celular também foi observada nas células nutridoras dos ovários meroísticos politróficos encontrados e descritos freqüentemente nos insetos, principalmente nos Hymenoptera (CAMARGO-MATHIAS, 1993). Nesse grupo de Arthropoda, as células nutridoras teriam a função de sintetizar e de enviar para o ovócito, elementos que participariam da composição final do vitelo, tal como aqui também demonstrado nas células do pedicelo dos ovários dos carrapatos. Nos ovários meroísticos, a degeneração das células nutridoras progride à medida que o ovócito vai se tornando maduro até chegar à degeneração total (CAMARGO-MATHIAS, 1993). Assim, diante de mais esse fato, o presente trabalho vem novamente confirmar a atuação do pedicelo como fonte de elementos para compor o vitelo dos ovócitos dos carrapatos.

As células do pedicelo que estão em contato direto com o ovócito IV de *A. triste* apresentaram núcleo com aspecto picnótico, citoplasma desorganizado e com a presença de grandes vacúolos, além da membrana plasmática sem microvilos no contato com o ovócito. Essas modificações nestas células do pedicelo indicariam o final do processo da vitelogênese e conseqüente final de transporte de material para o ovócito, permitindo confirmar que as especializações de membrana, representadas pelos microvilos, seriam umas das estruturas responsáveis pelo transporte de elementos exógenos vindos das células do pedicelo para os ovócitos e que o pedicelo, neste estágio já teria cumprido sua função de produzir e fornecer elementos para os ovócitos entrando, em virtude disso, num processo gradual de degeneração. No entanto, até que o ovócito torne-se maduro o suficiente para ser liberado para o interior do lúmen do ovário, o pedicelo persistirá fixando os mesmos.

O estudo citoquímico ultra-estrutural das células do pedicelo de *A. triste* mostrou que elas apresentam os polissacarídios no seu citoplasma independentemente do estágio em que o ovócito se encontra, sugerindo que este componente esteja sendo por elas produzidos e, em seguida, transportados para o ovócito através da sua captação pelos microvilos da membrana do ovócito via vesículas pinocíticas. No entanto, não fica descartada a hipótese de que possa estar ocorrendo síntese ativa de carboidratos no interior dos próprios ovócitos.

Polissacarídios, no entanto, não foram observados em todas as células de um mesmo pedicelo, ficando os mesmos mais evidentes apenas nas células em contato direto com o ovócito. Pode-se assim sugerir que as células do pedicelo trabalhariam assincronicamente na produção ou captação desse elemento, ou que apenas as células que estão em contato com o ovócito produziriam mais ativamente os polissacarídios para compor o vitelo. Camargo-Mathias (1993) sugeriu que em formigas *Neoponera villosa*, as células do epitélio folicular teriam participação passiva na deposição de polissacarídeos em ovócitos II, concordando com os dados de Ramamurty (1968), que sugeriu que açúcares como trealose, considerado o açúcar do sangue dos insetos, seriam transportados para o citoplasma periférico do ovócito por difusão

através do epitélio folicular. Camargo-Mathias et al. (1998) também observaram que, no diplopodo *Rhinocricus padbergi,* o epitélio folicular de ovócitos II participaria ativamente na síntese e/ou incorporação de polissacarídeos para o interior do ovócito.

Os lipídios também foram marcados citoquimicamente nas células do pedicelo dos ovários de *A. triste*, indicando que elas contribuem com fornecimento deste elemento para constituição do vitelo dos ovócitos, já que esse componente foi encontrado no citoplasma apical das células do pedicelo próximas do ovócito, bem como na região próxima às interdigitações entre as células do pedicelo, o que indicaria uma provável transferência de lipídios de célula para célula e finalmente das células para o interior do ovócito.

Em *A. triste*, as proteínas também foram evidenciadas nas células do pedicelo, cuja localização indicou que estas produzem e/ou incorporam proteínas vindas da hemolinfa, que posteriormente serão, ou transportadas para os ovócitos, ou por elas utilizadas com fins estruturais.

Através dos resultados obtidos no presente estudo, fica estabelecida a função do pedicelo como fornecedor de elementos de natureza polissacarídica, lipídica e protéica, para compor o vitelo dos ovócitos de carrapatos da espécie *A. triste*.

CONCLUSÕES

1. O ovário de carrapatos *Amblyomma triste* é do tipo panoístico.
2. À parede do ovário de *A. triste* estão presos ovócitos em quatro estágios de desenvolvimento através do pedicelo tendo os mesmos desenvolvimento sincrônico.
3. O vitelo de carrapatos *A. triste* é constituído por lipídios, proteínas e polissacarídios e que são depositados na seguinte seqüência: primeiramente os lipídios, em seguida as protéinas e por último, os polissacarídios.
4. Em carrapatos *A. triste*, os polissacarídios existentes nos ovócitos II, III e IV seriam produzidos exogeneamente ao ovário através de: a) produção e/ou transporte desse elemento via células do pedicelo e, b) captado da hemolinfa via membrana do ovócito.
5. Em carrapatos *A. triste*, os lipídios dos ovócitos têm origem: a) exógena, em todos os estágios, onde as células do pedicelo sintetizam e/ou os retiram da hemolinfa e, também através da captação deste elemento da

hemolinfa pelo próprio ovócito e, b) endógena, somente nos ovócitos III e IV, a partir das mitocôndrias.

6. Em carrapatos *A. triste*, as proteínas do vitelo dos ovócitos II, III e IV têm origem: a) exógena, via células do pedicelo que atuariam como fornecedoras deste elemento que seria produzido pelas mesmas e/ou retirado da hemolinfa e também por meio da captação da hemolinfa via membrana do ovócito e, b) endógena, apenas nos ovócitos IV.
7. A incorporação de elementos extra-ovarianos é contínua durante todo o processo de vitelogênese de *A. triste*.
8. As células do pedicelo de *A. triste* que estão fixando os ovócitos I e II são células com elevada atividade de síntese;
9. As células do pedicelo que fixam os ovócitos III de *A. triste* sofrem modificações que indicam início de processo de degeneração.
10. As células do pedicelo que estão em contato direto com o ovócito IV apresentam modificações que indicam o final do processo da vitelogênese e conseqüente final de transporte de material do pedicelo para o ovócito.
11. A participação das células do pedicelo no fornecimento de material vitelogênico para o ovócito de *A. triste* fica estabelecida.

REFERÊNCIAS BIBLIOGRÁFICAS

ANDERSON, JF; MAGNARELLI, LA. Vertebrate host relationships and distribution of ixodid ticks (Acari: *Ixodidae*) in Connecticut, USA. **Journal of Medical Entomology**, v. 17, p. 314-323, 1980.

AESCHLIMANN, A.; HECKER, H. Observations preliminaires sur l'ultrastructure de l'ovocyte en developpement chez *Ornithodoros moubata* Murray (Ixodidae: Argasidae). **Acta Tropical,** v. 25, p. 225-243, 1967.

BALASHOV, Y.S. A translation of bloodsucking ticks (Ixodidae) vectors of diseases of man and animals. **Miscellaneous Publications of the Entomological Society of America** 8, p. 159-376, 1972.

BALASHOV, Y.S. The female reproductive system. In: Balashov, Y.S. (Ed.), **An atlas of ixodid tick ultrastructure.** Russian: Entomological Society of America, 1983, p. 98-128.

BARNES, R.D.; RUPPERT, E.E. **Zoologia dos Invertebrados.** 6° edição. São Paulo: Roca Ltda, 1996. p. 642-651.

BECHARA, G.H. Carrapatos prejudicam carne. **Jornal da UNESP**, São Paulo, nº.175, 2003. p. 5.

BISHOP, F.C; HIXSON, H. Biology and economic importance of the Gulf Coast tick. **J. Econ. Entomol.**, v. 29, p. 1068-1076, 1936.

BOISSAN, L. **Gametogenese au cours du development pos embryonaire et biologie de la reproduction chez *Hysterochelifer meridianus* (L. Lock) Arachnidae: Pseudoscorpion**. Doctorat These, Facultat de Sc. De Montpelier, France, 1970.

BONHAG, P. F. Ovarian structure and vitellogenesis in insects. **Annual Review of Entomology,** v.3, p. 137-160, 1958.

BRINTON, L. P.; OLIVER, J. H. JR. Fine structure of oogonial and oocytes development in *Dermacentor andersoni* Stile (Acari: Ixodidae). **Journal of Parasitology,** v. 57, p. 720-747, 1971.

CAMARGO-MATHIAS, M.I. **Histoquímica e ultra-estrutura dos ovários de operárias e rainhas de formigas *Neoponera villosa* (Hymenoptera: Ponerinae).** 156p. Tese de doutorado, Instituto de Biociências, Universidade Estadual Paulista, Rio Claro, 1993.

CAMARGO-MATHIAS, M.I.; CAETANO, F. H. Ultrastructural study of the follicular epithelium in oocytes of *Pachycondyla* (*Neoponera) villosa* ants (Hymenoptera: Ponerinae). **Biocell**, v. 22, p. 53-60,1998.

CAMARGO-MATHIAS, M.I.; FONTANETTI, C.S.; MICÓ-BALAGUER, E. Histochemical studies of *Rhinocricus padbergi* Verhoeff ovaries (Diplopoda, Spirobolida, Rhinocricidae). **Cytobios,** v. 94, p. 169-184,1998.

CARVALHO, H.F.; RECCO-PIMENTEL, S.M. **A Célula 2001**. Barueri,: Manole Ltda, 2001. 287p.

CHAPMAN, F.R. **The Insects: Structure and Function**. 4ª. edição. New York: Cambridge University, 1998. 770 p.

CHARNIAUX-COTTON, H.; PAYEN, G. Crustacean Reproduction. In: LAUFER, H., DOWNER, R. G. (Eds.). **Endocrinology of selected invertebrate types**. New York: Alaw and Lius, 1985. p.279-300.

CONTI-DIAZ, I. A.; RUBIO, I.; SOMMA-MOREIRA, R. E.; PÉREZ-BORMIDA, G. Rickettsiosis cutáneo-ganglionar por *Rickettsia conorii* en el Uruguay. **Rev. Inst. Med. Tropical,** v. 32, n.5, p. 313-318, 1990.

CONTI-DÍAS, I. A. Rickettsiosis por *Rickettsia conoori* (fiebre botonosa del Mediterráneo o fiebre de Marsella). Estado actual en Uruguay. **Revista Medica Uruguay,** v.17, p.119-124, 2001.

CORREA, O. Carrapatos determinados no Rio Grande do Sul. Biologia, patologia e controle. **Boletim da Diretoria de Produção Animal,** v.10, n.18, p. 38-54, 1954.

CRUZ-LANDIM, C. Cell reorganization and cell death during the secretory cycle of the hypopharyngeal gland in Meliponinae bee workers (Hymenoptera: Apidae). **Acta Microscopica,** v.6, p.75-78., 1997.

DAVEY, R.B.; AHRENS, E.H.; GEORGE, J.E.; HUNTER,J.S; JAENNIN, P. Therapeutic and persistent efficacy of fipronil against *Boophilus microplus* (Acari: Ixodidae) on cattle. **Vet. Parasitol.,** v. 74, p. 261-276, 1998.

DEBRUYNE, M.; GUERIN, P.M. Isolation of 2,6-Dichlorophenol from the cattle tick *Boophilus microplus.* Receptor cell responses but no evidence for a behavioral response. **Journal Insect. Phys**., v. 40, p. 143-154. 1994.

DENARDI, S.E.; BECHARA, G.H.; OLIVEIRA, P.R.; NUNES, E.T.; SAITO, K.C.; CAMARGO-MATHIAS, M.I. Morphological characterization of the ovary and vitellogenesis dynamics in the *Amblyomma cajennense* (Acari: Ixodidae). **Veterinary. Parasitology,** v. 125, p.379-395, 2004.

DENARDI, S.E.; BECHARA, G.H.; CAMARGO-MATHIAS, M.I. Salivary gland cells of *Amblyomma cajennense* (Acari: Ixodidae) partially engorged females ticks. Lipids production by mitochondria. **Experimental Parasitology**, 2005. In press.

DIEHL, P. A. ZUR. Oogenese bei *Ornithodoros moubata* (Murray) (Ixodoidea: Argasidae) unter Besonderer Berücksichtigung der Vitellogenese. **Acta Tropical**, v. 27, p. 301-355, 1970.

DOUGLAS, J.R. The internal anatomy of *Dermacentor andersoni* Stiles. **Univ. Calif. Publ. Ent**., v.7, p. 202-272, 1943.

FLECHTMANN, C. H. W. **Ácaros de importância médico-veterinária**. São Paulo: Livraria Nobel S.A., 1973. 180p.

FREITAS, M.G. **Entomologia e Acarologia Médica e Veterinária**. Belo Horizonte: Precisa, 1982. 253p.

GRILLO, J.M. El problema de la resistencia a los acaricidas en los programas de control de la garrapata. **Bol. Ofic. Sanit. Panan.**, v.21, n. 3, p. 246-250, 1976.

HADDAD, A.; SESSO, A.; ATTIAS, M.; FARINA, M.; MEIRELLES, M.N.; SILVEIRA, M.; BENCHIMOL, M.; SOARES, M.J.; BARTH, O.M.; MACHADO, R.D.; SOUTO-PADRÓN, T.; SOUZA, W. **Técnicas Básicas de Microscopia Eletrônica aplicadas às Ciências Biológicas**. In: WANDERLEY DE SOUZA (Org), 1998. p. 66-73.

HARWOOD, R.F.; JAMES,M.T. **Entomology in human and animal health**. 7 ° edição, New York: Macmillan Publishin Co., 1979. 548p.

HINTON, H. E. **Biology of Insect Egg Shells**. Oxford: Pergamon, 1982. 212p.

HOOGSTRAAL, H. African Ixodoidea I. Ticks of the Sudan. **Res. Rep. U. S. Naval Med. Res. Unit**., n.3, p. 1000-1101, 1956.

IVANCOVICH, J.C. Reclasificación de algunas especies de garrapatas del género *Amblyomma* (Ixodoidea) en la Argentina. **Investigaciones Agropecuarias,** v. 15, p. 673-682, 1980.

JUNQUEIRA, L.C.U.; JUNQUEIRA, L.M.M.S. **Técnicas básicas de citologia e histologia**. São Paulo: Livraria Editora Santos, 1983. p. 48-81.

KEIRANS, J.E. George Henry Faulkner Nuttall and the Nuttall tick catalogue. **United States Department of Agriculture Research Series Miscelaneous Publications**, v. 1438, p. 1780-1785, 1984.

KING, R.C. Oogenesis in adult *Drosophila melanogaster* IX. Studies on the cytochemistry and ultrastructure of developing oocytes. **Growth**, v. 24, p. 265-323, 1960.

KOHLS, G.M. Concerning the identity of *Amblyomma maculatum, Amblyomma tigrinum, Amblyomma triste* and *Amblyomma ovatum* of Koch, 1844. **Proceedings Of The Entomological Society of Washington,** v.58 ,n. 3, p.143-147,1956.

LEMOS, E.R.S.; MACHADO, R.D.; COURA, J.R.; GUIMARÃES, M.A.; FREIRE, N.M.; AMORIM, M.; GAZETA, G.S. Epidemiological aspects of the Brazilian spotted fever: seasonal activity of ticks collected in an endemic area in São Paulo. **Revista da Sociedade Brasileira de Medicina Tropical,** v.3, n. 3, p. 181-185, 1997.

LIPA, J.J. Microbial control of mites and ticks. In: BURGES, H.D., HUSSEY, N.W. (Eds). **Microbial control of insects and mites**. 2°edição. London: Academic, 1971. p. 357-374.

LISON, L. **Histochimie et cytochimie animales**. 3° edição. Paris: Gauthier Villars, 1960. 397p.

MCMULLEN, H.L.; SAUER, J.R.; BURTON, R.L. Possible role in uptake of water vapour by ixodid tick salivary glands. **Journal of Insect Physiology**, v.22, p. 1281-1285, 1976.

MOORHOUSE, D.E.; TATCHELL, R.J. The feeding process of the cattle tick *Boophilus microplus* (Canestrini). A study in host-parasite relations. Attachment to the host. **Parasitology,** v.56, p. 623-632, 1966.

NEEDHAM, G.R.; ROSELL, R.; GREENWALD, L.; COONS,L.B. Ultrastructure of type I salivary gland acini in four species of ticks and the influence of hydration states on type I acini of *Amblyomma americanum*. **Experimental and Applied Acarology**, v. 10, p.83-104, 1990.

NEEDHAM, G.R.;TEEL, P.D. Off host physiological ecology of Ixodid ticks. **Annual Review of Entomology,** v. 36, p. 659-681, 1991.

NUNES, E.T.; BECHARA, G.H.; SAITO, K. C.; DENARDI, S.E.; OLIVEIRA, P.R.; CAMARGO-MATHIAS, M.I. Morphological, histological, and ultrastructural characterization of degenerating salivary glands in females of the cattle-tick *Rhipicephalus (Boophilus) microplus* (CANESTRINI, 1887) (Acari: Ixodidae). **Micron,** v.36, p. 433-447, 2005.

NEWTON, L.G. Acaricide resistence and cattle tick control. **Aust. Vet. J**., v. 43, n. 9, p. 389-394, 1967.

NORREVANG, A. Electron microscopic morphology of oogenesis. **International Review of Cytology**, v. 23, p. 114-186, 1968.

OLIVEIRA, P.R.; BECHARA, G.H.; DENARDI, S.E.; SAITO, K.C.; NUNES, E.T.; CAMARGO-MATHIAS, M.I. Morphological characterization of the ovary and oocytes vitellogenesis of the tick *Rhipicephalus sanguineus* (LATREILLE, 1806) (Acari: Ixodidae). **Experimental Parasitology,** v. 110, p.146-156, 2005.

OLIVEIRA, P.R.; CAMARGO-MATHIAS, M.I.; BECHARA, G.H. Females Ticks of *Amblyomma triste* (Kock, 1844) (Acari: Ixodidae). Ovaries description and vitellogenesis process. **Experimental Parasitology**, 2005. In press.

OLIVEIRA, P.R.; CAMARGO-MATHIAS, M.I.; BECHARA, G.H. Female Ticks of *Amblyomma triste* (Koch, 1844) (Acari: Ixodidae). Ovaries ultrastructural analysis. **Micron**, 2005. In press.

PEARSE, A.G.E. **Histochemistry theoretical and applied**. 2 º edição. Churchill: Livingstone, 1985. 449p.

PETER, O.; BROSSARD, M. Tick control. **Medicine et maladies infectieuses**, v. 28, p. 383-386, 1998.

RAMAMURTY, P.S. Origin and distribution of glycogen during vitellogenesis of the scorpion fly *Panorpa communis*. **Journal Insect Physiology**, v. 14, p. 1325-1330,1968.

RANADE, V. On the cytoplasmic inclusions in the oogenesis of *Periplaneta Americana* Linn. Allahad. **University Science Bulletin**, v. 9, p. 85-121, 1933.

REDDY, J. T.; LOCKE, M. The size limited penetration of gold particles through insect basal laminae. **J. Insect Physiol.,** v. 36, n**.** 6, p. 397-399, 1990.

REY,L. **Parasitologia.** São Paulo: Guanabara Koogan,. 1973. 212p.

REY, L. **Parasitos e Doenças Parasitárias do Homem nas Américas e na África**. Rio de Janeiro: Guanabara Koogan, 2001. p. 752-759.

RIBEIRO, J.M.C. Role of saliva in blood-feeding by arthropods. **Annals of Review Entomology**, v.32, p. 463-478, 1987.

ROBINSON, L.E.; DAVIDSON, L.E. The anatomy of *Argas persicus* II. **Parasitology**, v. 6, p. 217-256, 1913.

ROSELL, R.; COONS, L.B. The role of the fat-body, midgut and ovary in vitellogenin production and vitellogenesis in the female tick, *Dermacentor variabilis*. **International Journal for Parasitology**, v. 22, p. 341-349, 1992.

ROSS, I.C. An experimental study of tick paralysis in Australia. **Parasitology**, v. 18, p. 410-29, 1926.

RUDOLPH, D.; KNULLE, W. Site and mechanism of water vapour uptake from the atmosphere of ixodid ticks. **Nature**, v. 249, p. 84-85, 1974.

SAID, E.A. A contribution to the anatomy and histology of the female reproductive system of *Amblyomma cajennense* (Acarina : Ixodidae). **Journal of the Egyptian Society of Parasitology**, v. 22, p. 385-393, 1992.

SAITO, K. C.; BECHARA, G.H.; OLIVEIRA, P.R.; NUNES, E.T.; DENARDI, S.E.; CAMARGO-MATHIAS, M.I. Morphological, histological, and ultrastructural studies of the ovary of the tick *Boophilus microplus* (CANESTRINI, 1887) (Acari: Ixodidae). **Veterinary Parasitology,** v.129, p. 299-311, 2005.

SAUER, J.R.; ESSENBERG, R.C.; BOWMAN, A.S. Salivary glands in ixodid ticks: control and mechanism of secretion. **Journal of Insect Physiology**, v. 46, p. 1069-1078, 1999.

SEMTNER, P.J.; HAIR J.A. Distribution, seasonal abundance, and hosts of the Gulf Coast tick in Oklahoma. **Annais Entomological Soc. Amer.,** v. 66, p. 1264-1268, 1973.

SIGAL, M.D.; YODER, J.A.; NEEDHAM, G.R. Palp-splaying behavior and a specific mouthpart site associated with active water vapor uptake in *Amblyomma americanum* (Acari: Ixodidae). **Journal of Medical Entomology**, v. 36, p. 365-369, 1999.

SINKOC, A.L.; BRUM, J.G.W.; MÜLLER, G.; BEGROW, A.; PAULSEN, R.M.M. Ixodideos parasitos de capivaras (*Hydrochaeris hydrochaeris* L., 1766) da Estação Ecológica do Taim, Rio Grande, RS. **Ciência Rural**, v. 27, n. 1, p.119-122, 1997.

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

STORER, T.I.; USINGER, R.L. **Zoologia Geral**. 3° edição. São Paulo: Companhia ed. Nacional, 1977. p. 523-524.

SZABÓ, M. P. J.; LABRUNA, M. B.; PEREIRA, M. C.; DUARTE, J. M. B. Ticks (Acari: Ixodidae) on Wild Marsh-Deer (*Blastocerus dichotomus*) from Southeast Brazil: Infestations before and after habitat loss. **Journal of Medical Entomology**, v. 40, n. 3, p. 268–274, 2003.

TATCHELL, R.J.; MOORHOUSE, D.E. The feeding process of the cattle tick *Boophilus microplus* (Canestrini). The sequence of host-tissue changes. **Parasitology,** v. 58, p. 441-459, 1970.

TELLAM, R.L.; SMITH, D.; KEMP, D. H. Vaccination against ticks. In: YONG, W. K. (Ed.). **Animal parasite control using biotechnology**. Boca Raton: CRC Press, 1992. p. 303-331.

TILL, W.M. A contribution to the anatomy and histology of the brown ear tick *Rhipicephalus appendiculatus*. **Mem. Entomol. Soc. Southern Africa**, v. 6, p.1-124, 1961.

VENZAL, J. M.; FREGUEIRO, G. Ixódidos parásitos de marsupiales (*Didelphimorphia: Didelphide*) y roedores (*Rodentia: Muridae*) de la fauna uruguaya . **Rev. Facultad de Veterinaria**, p. 9-11. 1999.

VENZAL, J.M.; GUGLIELMONE, A.A.; ESTRADA-PENA, A.; CABRERA, P.A.; CASTRO, O. Ticks (Ixodida: Ixodidae) parasitising humans in Uruguay. **Ann Tropical Medicine Parasitology**, v. 97, n. 7, p. 769-772, 2003.

VENZAL, J.M; PORTILLO, A.; ESTRADA-PENA, A.; CASTRO, O.; CABRERA, P.A.; OTEO, J.A. *Rickettsia parkeri* in *Amblyomma triste* from Uruguay. **Emerging Infectious Diseases**, v.10, n.8, p. 1493-1495, 2004.

VITZTHUM, H. G. Acarina. In: ______. (Org). **Brown's Klassen und Ordnungen des Tierreiches**. Leipzig: Becker u. Erler, 1943. p. 35-62.

WALKER, A. **Arthropods of domestic animals. A guide to preliminary identification**. London: Chapman & Hall, 1994. p. 5-60.

WALL, R.; SHEARER, D. **Veterinary Entomology**. London: Chapman & Hall, 1997. p. 43-139.

WIGGLESWORTH, V. B. The hormonal regulation of growth and reproduction in insects. In: BEAMENT, J. W. L., THEHERNE, J. E., WIGGLESWORTH, V. B. (Eds). **Advances in Insect Physiology**. New York: Academic Press, 1964. p. 247-336.

WILLADSEN, P. Novel vaccines for ectoparasites. **Veterinary Parasitology**, v. 71, p. 209-222, 1997.

ZUMPT,F.; GLAJCHEN,D. Tick paralysis in man. **S. Afr. Med. J.,** v.24, p. 1092-94, 1950.

Orientadora	Orientada
Maria Izabel Camargo Mathias	Patrícia Rosa de Oliveira