UNIVERSIDADE ESTADUAL DE CAMPINAS

ADELINA APARECIDA FRANCISCA FERREIRA

Ciclo Reprodutivo e Espermiogênese de *Iguana iguana* (Reptilia: Sauria: Iguanidae)

Tese apresentada ao Instituto de Biologia para a obtenção do título de Mestre em Biologia Celular e Estrutural, na área de Histologia.

Orientadora: Prof[a]. Dr[a]. Mary Anne Heidi Dolder

FICHA CATALOGRÁFICA ELABORADA PELA BIBLIOTECA DO INSTITUTO DE BIOLOGIA - UNICAMP

Ferreira, Adelina Aparecida Francisca

F414c Ciclo reprodutivo e espermiogênese de *Iguana iguana* (Reptilia: Sauria: Iguanidae)/Adelina Aparecida Francisca Ferreira. -- Campinas, SP.[s.n.], 2001

F413c

71f: ilus.

Orientadora: Mary Anne Heidi Dolder

Dissertação (mestrado) – Universidade Estadual de Campinas. Instituto de Biologia.

1. Reprodução. 2. Espermiogênese. 3. Lagartos. 4. Pantanal. I. Dolder, Mary Anne Heidi. II. Universidade Estadual de Campinas. Instituto de Biologia. IV. Título.

Data da Defesa: 18/09/2001

BANCA EXAMINADORA

Prof[a]. Dr[a]. Mary Anne Heidi Dolder (Orientadora) _____
Assinatura

Prof[a]. Dr[a]. Shirlei Maria Recco Pimentel _____
Assinatura

Prof. Dr. Carlos Alberto Vicentini _____
Assinatura

Prof[a]. Dr[a]. Maria Alice da Cruz Höfling _____
Assinatura

Dedico

Aos meus amados pais,

Meus avós

E aos anjos...

"...Mas os que confiam no Senhor renovam suas forças, sobem com asas como águias, correm e não se cansam, caminham e não se fatigam."

Isaías 40:31

Agradeço

À Jesus, à Maria e aos Anjos.

Aos meus pais pelo exemplo de atitudes e incentivo.

À Prof[a]. Dr[a]. Heidi Dolder pela orientação e agradável amizade.

Aos Profs. Shirlei Recco-Pimentel, Carlos Alberto Vicentini e Maria Alice Cruz Höfling pela análise prévia da tese.

À todos os professores dos Departamentos de Biologia Celular, Histologia e Anatomia (UNICAMP) pelo apoio no meu crescimento intelectual e profissional.

Aos amigos de laboratório Karina, Uyrá, Cláudia e Lino pela acolhida e ajuda.

Aos amigos e colaboradores Iraceles Laura, Paulo Robson de Souza e Lúcio Viana da Universidade Federal de Mato Grosso do Sul.

Ao Instituto Brasileiro de Meio Ambiente (IBAMA) por fornecer a autorização para coleta e transporte dos espécimes.

À coordenação da Base de Estudos do Pantanal (BEP/UFMS) pelo apoio técnico nas atividades de campo.

Às técnicas do Centro de Microscopia Eletrônica da UNICAMP, pela presteza.

À Fundação de Apoio a Pesquisa do Estado de São Paulo (FAPESP) pela concessão da bolsa.

E a todos aqueles que direta ou indiretamente auxiliaram no desempenho de minhas atividades.

Índice

1. RESUMO

Espécimes adultos (N = 10) do lagarto *Iguana iguana* foram coletados no Pantanal brasileiro, com o objetivo de descrever o ciclo reprodutivo anual e o processo de espermiogênese. Os testículos foram removidos e processados através de técnicas convencionais para microscopia de luz e eletrônica. Durante o ciclo reprodutivo anual foram observadas quatro fases da espermatogênese: 1° estadio (outubro a dezembro), divisões iniciais de espermatogônias, sendo o tecido intersticial e células de Leydig extremamente evidentes; 2° estadio (janeiro a março), período de reduzida proliferação da linhagem germinativa ou quiescência testicular e início da redução do tecido intersticial; 3° estadio (abril a junho) desenvolvimento máximo das células germinativas, com todos os graus de maturidade celular, enquanto o tecido intersticial é reduzido; 4° estadio (julho a setembro) a espermiação já ocorreu e encontra-se citoplasma residual no lumen dos túbulos seminíferos. No processo de espermiogênese são formados grupos de estruturas, como o complexo acrosomal na cabeça, e o complexo axonêmico nas peças intermediária, principal e terminal. O núcleo sofre uma compactação da cromatina, inicialmente em filamentos grossos e longitudinais. A forma do núcleo é determinada pela compactação e pela influência dos microtúbulos periféricos e circulares, a "manchette". O complexo acrosomal é formado à partir da fusão de vesículas produzidas pelo complexo de Golgi, e pela a interação da vesícula pró acrosômica e o núcleo. A peça intermediária apresenta um par de centríolos, proximal e distal que são rodeados por uma bainha fibrosa e anéis de mitocôndrias simples e modificadas. Os centríolos sustentam o axonema do flagelo que é formado na última porção da peça intermediária. O flagelo é formado pela peça principal, com o axonema no padrão 9 + 2 e cercado pela bainha fibrosa, enquanto na peça terminal, o axonema se mantém, sendo rodeado apenas pela membrana plasmática. Concluímos com esses dados que o lagarto *I.* iguana apresenta um período reprodutivo curto e especialmente adaptado as condições climáticas do Pantanal; os espermatozóides são semelhantes aos observados em vários outros lagartos, no entanto são considerados imaturos morfologicamente no momento da espermiação, pela presença de citoplasma em torno da cabeça e também de corpos densos pouco evidentes na peça intermediária.

2. ABSTRACT

Adult specimens (N = 10) of the lizard *Iguana iguana* were collected in the Brazilian Pantanal with the objective of describing the annual reproductive cycle and the process of spermiogenesis. The testes were removed and processed with conventional techniques for light and electron microscopy. During the annual reproductive cycle four phases of spermatogenesis were observed. Stage 1 (October-December): Initial divisions of spermatogonia occur, while interstitial tissue and Leydig cells are extremely evident. Stage 2 (January-March): A period of reduced cell proliferation or testicular quiescence and inicial reduction of the interstitial tissue. Stage 3 (April-June): Maximum development of the germ cells, with all phases of cellular maturity, while the interstitial tissue is greatly reduced. Stage 4 (July-September): Spermiation has already occurred and residual cytoplasm is found in the seminiferous tubule lumen. In the process of spermiogenesis groups of structures are formed, such as the acrosomal complex in the head, and the axonemic complex in the middle, principal and terminal piece. The nucleus is compacted, initially into thickening helicoidal chromatin filaments, which later extend longitudinally. The chromatin condensation and the influence of the microtubule sheath, in the form of a "manchette" determine the nuclear shape. The acrosomal complex is formed by the fusion of Golgian vesicles, followed by interaction of the pro-acrosomic vesicle and the nucleus. The middle piece presents the proximal and distal centrioles that are surrounded by a fibrous sheath and rings of simple and modified mitochondria. The centrioles sustain the axoneme at the final portion of the flagellar middle piece. The flagellum is formed by the principal piece, with an axoneme in the standard 9 + 2 microtubule arrangement, surrounded by a fibrous sheath and the terminal piece, where the axoneme is encircled only by the plasma membrane. With this data, we conclude that the lizard, *I. Iguana,* has short reproductive period specially adapted to the climatic conditions of the Pantanal. The spermatozoon is similar to that of other lizards, however it was considered morphologically immature at spermiation, because of the presence of cytoplasm around the head, as well as developing dense bodies.

3. INTRODUÇÃO

3.1. CARACTERIZAÇÃO DA ESPÉCIE

O lagarto *Iguana iguana* (Lineu - 1758) pertence à Família Iguanidae, Subordem Sauria, Ordem Squamata, Infraclasse Lepidossauria, Subclasse Diapsida e Classe Reptilia do Reino Animal. Esta família reúne cinco Subfamílias, 54 Gêneros e mais de 700 Espécies tropicais e subtropicais (Zug, 1993). A espécie *I. iguana* é comum em regiões neotropicais, com ampla distribuição do México ao Brasil Central (Robinson & Redford, 1991). Apresenta características marcantes e exclusivas (Fig. 1), como uma crista dorsal que vai da nuca à cauda, uma grande escama redonda em baixo do tímpano e uma crista gular. Possui a cabeça curta, rostro arredondado, narina grande, e tímpano grande e oval. As escamas da cabeça são lisas e de tamanhos variáveis. As escamas dorsais são muito pequenas, iguais e quilhadas. O pescoço possui tubérculos grandes, cônicos e quilhados, irregularmente distribuídos. As escamas ventrais são maiores que as dorsais e lisas. A cauda é longa e comprimida, com escamas quilhadas, maiores ventralmente. Apresenta um colorido verde, ventralmente mais claro, e as superfícies dorsais variando entre mais claro e escuro, podendo mudar e aproximar-se da cor do ambiente (Vanzolini *et al.* 1980). Os membros são fortes, dígitos longos e finos, com garras. Apresentam de 12 a 20 poros femorais, ventralmente dispostos em cada membro pélvico, cuja secreção nos machos é basicamente constituída de hormônios e feromônios, com função de demarcação de território e atrativo às fêmeas no período reprodutivo (Alberts *et al.*, 1992). A idade média destes animais é de 15 anos. Os filhotes medem aproximadamente 30 cm e o adulto pode chegar a 2 metros da cabeça à cauda. São arborícolas, territorialistas e polígamos (Bock & Rand, 1989; Pratt *et al.*, 1994). Eventualmente nadam, e no chão correm com velocidade, porém por pouco tempo e curtas distâncias. Aparentemente são herbívoros, no entanto alguns insetos foram encontrados em seu estômago, sem se saber se a deglutição foi ou não voluntária junto à folhagem (Vanzolini *et al.*, 1980). A postura, cerca de 30 a 50 ovos (Hirth, 1963; Klein, 1982; Drumond & Burghardt, 1983), é enterrada na areia (Rand & Dugan, 1980; Brust, 1985), a alguns quilômetros da mata ciliar no Pantanal brasileiro (observações pessoais).

3.2. REPRODUÇÃO DE LAGARTOS X SAZONALIDADE

É fato conhecido que o ciclo reprodutivo nos diferentes animais está sob controle hormonal (Reddy & Prasad, 1970). Entretanto, nos lagartos os fatores extrínsecos também influenciam o ciclo como reguladores (Fox & Dessauer, 1957; Andrews, 1982; Rand & Bock, 1992). Alguns fatores podem ser enumerados:

3.2.1. FATORES INTRÍNSECOS

O controle hormonal é um fator intrínseco, cujo mecanismo de atuação das gonadotrofinas já foi bastante explorado para lagartos por Young *et al.* (1995a) e intensamente para vários mamíferos. No início do período de crescimento gonadal, durante o ciclo anual, a concentração de gonadotrofinas circulantes aumenta, em conseqüência do aumento dos impulsos externos como o aumento da temperatura, fotoperíodo e pluviosidade (Reddy & Prasad, 1970). A testosterona aumenta progressivamente nos machos em resposta à secreção de gonadotrofinas e aumenta conforme se aproxima do período de espermiação. O aumento do nível sangüíneo de testosterona provoca também o final da secreção de gonadotrofinas, por um efeito retrógrado, o que vai provocar o esvaziamento dos túbulos após a espermiação e a entrada no período de repouso (Young *et al.*, 1995b).

No entanto, em lagartos existe um hormônio que exerce forte influência no ciclo gonadal: a melatonina. Este é um hormônio elaborado pela glândula pineal e derivado de um aminoácido essencial, o triptofano. A Pineal teve suas propriedades biquímicas conhecidas no final do século passado e foi apontada como o "terceiro olho" das aves (Haldar & Thapliyal, 1980). A glândula pineal é, também para os lagartos, um órgão fotorreceptor. Esta glândula, através do nervo óptico, é capaz de informar às partes internas do organismo sobre as condições de iluminação do ambiente. Desta forma, de acordo com a intensidade de luz que recebe, a glândula pineal libera no organismo a melatonina (Berthelot *et al.*, 1991). Nos lagartos esse hormônio sinaliza as mudanças sazonais, podendo estimular ou inibir o "crescimento" gonadal (Dekoninck, 1991). Essa característica é observável de forma mais determinante no ciclo gonadal de lagartos em regiões temperadas, cujas estações climáticas são bem definidas. Não é objetivo desta tese explorar este aspecto, no entanto é interessante saber que foi verificada uma intensa ligação entre os fatores hormonais e ambientais influenciando o ciclo reprodutivo.

3.2.2. FATORES EXTRÍNSECOS

Os padrões de fotoperíodo, além de temperatura e pluviosidade, funcionam como estímulos extrínsecos (Bartholomew, 1950; Fox & Dessauer, 1957; Licht, 1967a; 1967b). Estes levam consigo um ritmo endógeno, ou então, acionam diretamente as modificações fisiológicas do sistema reprodutor durante a estação reprodutiva. Estes fatores externos também são influenciados por mecanismos endócrinos e neuroendócrinos, assim são equilibrados contínua e reciprocamente.

Não existe um padrão de fotoperíodos e temperaturas ideais para desencadear a espermatogênese. Esses valores variam de acordo com a espécie e o local onde habitam. Para os lagartos *Anolis carolinensis* (Licht, 1967 e 1970), *Cnemidophorus leminicatus* (Del Conte, 1972) em regiões temperadas, além de *Sceloporus mucronatus* (Mendez de La Cruz *et al.*, 1988), *Barisia imbricata* (Guillete *et al.*, 1987) e *Sceloporus torquatus* (Guillette *et al.*, 1993) em regiões tropicais, o comprimento de fotoperíodo varia em torno das 12 horas. As temperaturas variam em torno dos 20°C nos países temperados, e em torno dos 15°C em países tropicais. Estes dados confirmam que em países tropicais a temperatura não desempenha função desencadeante, visto que esta condição climática é relativamente constante. A pluviosidade é o fator determinante. Estas variações demonstram o potencial adaptativo destes animais.

Em várias espécies de lagartos, em especial os habitantes de regiões temperadas, a variação no fotoperíodo é o principal estímulo ambiental, na sincronização da reprodução com a sazonalidade (Licht, 1970). A variação na temperatura ambiental pode influenciar e até modificar o efeito estacional do fotoperíodo sobre a função reprodutiva (Fox & Dessauer, 1957; Licht, 1970). O ciclo de machos e fêmeas de lagartos parece estar adaptado às condições de temperatura necessárias ao desenvolvimento do embrião e crescimento do recém nato (Fitch, 1970).

3.2.3. FATORES INTRÍNSECOS X FATORES EXTRÍNSECOS

Os efeitos do fotoperíodo envolvem, pelo menos, dois mecanismos separados. Primeiro existe uma ação direta do eixo hipófise - hipotálamo. Os níveis de gonadotrofinas atingem um nível máximo durante o período reprodutivo, e diminuem durante o período de repouso. Secundariamente, existe uma modificação simultânea, na sensibilidade do sistema nervoso central ao mecanismo retrógrado dos esteróides. Impulsos nervosos são gerados,

em conseqüência de variações na incidência de luz sobre os fotorreceptores da retina. Esses impulsos luminosos são transmitidos para a glândula pineal, que comanda a síntese e a secreção de melatonina, iniciando a cascata de eventos que induzem a atividade gonadal (Haldar & Thapliyal, 1980; Young, 1995a ; 1995b).

Portanto, é considerável a importância da luz solar e da temperatura em relação à periodicidade na reprodução de lagartos, observados também em estudos experimentais (Bartholomeu, 1953; Licht, 1970). Entretanto, há muitos outros fatores que influenciam a atividade gonadal, como precipitação pluviométrica, suprimento alimentar, predação, formação de casais e territorialismo (Fitch, 1940; Rand & Bock, 1992). É a união desses vários fatores controladores às características adaptativas de cada espécie de lagarto que determina quando e como ocorre a gênese dos gametas, principalmente em machos.

3.3. CICLO REPRODUTIVO DE LAGARTOS

O processo de divisão, proliferação e maturação das células germinativas segue um ciclo anual bem definido em todos os lagartos. As variações observadas entre as diferentes espécies dependem basicamente dos fatores externos que exercem maior influência, e do potencial adaptativo de cada espécie a esses fatores externos.

Para vários autores (Bartholomew, 1950; Fox & Dessauer, 1957; 1958; Wilhoft & Quay, 1961; Wilhoft, 1963; Hahn, 1964; Goldberg & Lowe, 1966; Licht, 1967; Rand & Bock, 1992) o estímulo da variação de temperatura anual é o fator desencadeante da atividade testicular. Geralmente, o aumento da temperatura ambiental associada a um aumento do fotoperíodo, característicos do verão, são propícios para o período de reprodução. Essa característica é marcante para lagartos de clima temperado (Sanyal & Prasad, 1965; 1967; Childress, 1970; Licht, 1970; Mayhew & Wright, 1970; Ballinger & Nietfeldt, 1989; Gavaud, 1991). Em regiões de clima tropical o ciclo reprodutivo de lagartos também apresenta características semelhantes, no entanto o fator externo mais preponderante é a variação pluviométrica e a disponibilidade de alimento (Goldberg, 1970; Marion & Sexton, 1970; Licht & Gorman, 1975; Ortega & Barbault, 1984; Guillette & Sullivan, 1985; Guillette & Casas-Andreu, 1987; Ramirez-Pinilla, 1991).

Portanto, o ciclo reprodutivo de lagartos é definido pela união de fatores externos, que influenciam diferentemente a atividade fisiológica, estimulando ou inibindo o período

de reprodução. Cada espécie desenvolve uma adaptação ao ambiente de acordo com a preponderância de determinados estímulos.

Essa variação de ciclos, para locais com condições climáticas diferentes, pode ser observada dentro de várias espécies (Hirth, 1963; Licht, 1970; Dugan, 1982; Klein, 1982; Pratt *et al.*, 1994). Por essas razões é importante avaliar o ciclo reprodutivo de uma mesma espécie em diferentes locais, tanto para efetuar uma análise comparativa, quanto para iniciar outros estudos mais detalhados como, por exemplo, a espermiogênese.

3.4. ESPERMIOGÊNESE EM LAGARTOS

Apesar do processo de espermiogênese ser relativamente semelhante entre os Iguanídeos: *Anolis carolinensis* (Guraya, 1971) *Tropidurus torquatus* (Cruz-Landim & Cruz-Höfling, 1977) *Iguana delicatissima* (Saita *et al.*, 1988) e os Lacertídeos: *Calotes versicolor* (Ghasay & Bandapadhyay, 1983), *Lacerta vivipara* (Courtens & Depeiges, 1985). Existem pequenas modificações que possibilitam observar diferenças entre as espécies. As semelhanças e diferenças observadas entre os lagartos *Podarcis taurica* (Butler & Gabri, 1984), *Agama stellio* (Al-Hajj *et al.*, 1987), *Cnemidophorus sexlineatus* (Newton & Trauth, 1992); Sphenodontes (Healy & Jamieson, 1994); Serpentes (Hamilton & Fawcett, 1968; Al-Dokhi, 1997); Crocodilianos (Jamieson *et al.*, 1997); Chelônios (Yasuzumi & Yasuda, 1968); assim como em Aves (Asa *et al.*, 1986; Sprando & Russel, 1988) e Mamíferos (Fawcett & Phillips, 1970; Fawcett *et al.*, 1971; Phillips, 1972; Baccetti *et al.*, 1980) demonstram que a ultra-estrutura do espermatozóide pode ser uma importante informação para a filogenia destes grupos (Baccetti, 1986).

O estudo da espermiogênese pode auxiliar no entendimento da funcionalidade de determinadas porções do espermatozóide, como também do momento em que esta célula está apta para desempenhar sua função de fecundar o óvulo. O espermatozóide nem sempre encontra-se "maduro" no testículo. As modificações posteriores desta célula em seu percurso nos canais condutores do macho e até no receptáculo seminal da fêmea, geralmente são em nível de receptores de membrana (Conner and Crews, 1980; Bouresli *et al.*, 1981; Halpert *et al.*, 1982; Adams and Cooper, 1988; Srinivas *et al.*, 1995) e não foram registradas mudanças na morfologia do espermatozóide em Lacertílios. Devemos considerar que o grupo Squamata reúne um número relativamente grande de espécies, as

quais, na sua maioria, não foram estudadas e pouco se sabe sobre as diferenças e/ou semelhanças existentes dentro deste grupo quanto ao ciclo reprodutivo e formação de gametas. Esses estudos podem oferecer importantes informações para estudos taxonômicos e/ou filogenéticos.

Figura 1: Macho adulto de *Iguana iguana.*

4. OBJETIVOS

O presente trabalho tem o propósito de descrever os processos de espermatogênese e espermiogênese durante o ciclo reprodutivo anual de *I. iguana* e verificar se há correlações com variações climáticas do Pantanal Sul Matogrossense.

As informações de espermatogênese foram colhidas com a intenção de contribuir para trabalhos futuros de ecologia, etologia e preservação que dependam do conhecimento do ciclo reprodutivo de *I. iguana* na região do Pantanal. Trabalhos de sistemática e filogenia podem ser beneficiados com os dados descritos da espermiogênese tanto para a família Iguanidae como para outros Répteis.

5. REFERÊNCIAS BIBLIOGRÁFICAS

Alberts, A. C.; Sharp, T. R.; Werner, D. I. & Weldon, P. J. 1992. Seasonal variation of lipids in femoral gland secretions of male green iguanas (*Iguana iguana*). **J. Chem. Ecol. 18:** 703-712.

Al-Dokhi, O. A. 1997. Fine structure of sperm tail differentiation in the sand skink, *Scincus mitranus* (Anderson, 1871) (Squamata: Reptilia). **J. Arid Envirom. 37:** 395-401.

Al-Hajj, H.; Janakat, S. & Mahmoud, F. 1987. Electron microscopic study of sperm head differentiation in the lizard *Agama stellio*. **Can. J. Zool. 65:** 2959-2968.

Andrews, R. M. 1982. Patterns of growth in Reptile. *In:* Gans, C. & Pough, F. H. (edits) **Biology of the Reptilia, vol. 13, Physiology D**, p: 273-320. Academic Press.

Asa, C. S.; Phillips, D. M. & Stover, J. 1986. Ultrastructure of spermatozoa of the crested tinamou. **J. Ultrastruct. Res. 94:** 170-175.

Baccetti, B. 1986. Evolutionary trends in sperm structure. **Comp. Biochem. Physiol. 85:** 29-36.

Baccetti, B.; Bigliardi, E. & Burrini, A. G. 1980. The morphogenesis of vertebrates perforatorium. **J. Ultrastruct. Res. 71:** 272-287.

Ballinger, R. E. & Nietfeldt, J. W. 1989. Ontogenetic stages of reproductive maturity in the viviparous lizard, *Sceloporus jarrovi* (Iguanidae). **J. Herpetol. 23:** 282-292.

Bartholomeu, G. A. 1950. The effects of artificially controlled temperature and day length on gonadal development in a lizard, *Xantusia vigilis*. **Anat. Rec. 106:** 49-59.

Bartholomeu, G. A. 1953. The modification by temperature of photoperiodic control of gonadal development in the lizard *Xantusia vigilis*. **Copeia 1953:** 45-50.

Berthelot, X.; Neuhart, L. & Gay, F. 1991. Photoperiodicity, melatonin and reproduction in cows. **Recueil Med. Vet. 167:** 219-225.

Bock, B. C. & Rand, A. S. 1989. Factors influencing nesting synchrony and hatching sucess at a green iguana nesting aggregation in Panama. **Copeia 1989:** 978-986.

Bouresli, M. N.; Bishay, L. F. & Alzaid, N. S. 1981. Observations on the fine structure of the sperm storage crypts in the lizard *Acanthodactylus scutellatus* Hardyi. **Arch. Biol. 92:** 287-298.

Brust, D. G. 1985. The physiological and ecological consequences of burrow-dwelling to the green iguana (*Iguana iguana*). **Amer. Zool. 25:** 7A.

Butler, R. D. & Gabri, M. S. 1984. Structure and development of the sperm head in the lizard *Podarcis* (Lacerta) *taurica*. **J. Ultrastruct. Res. 88:** 261-274.

Childress, J. R. 1970. Observations on the reproductive cycle of *Agama stellio picea*. **Herpetologica 26:** 149-155.

Conner, J. & Crews, D. 1980. Sperm transfer and storage in the lizard *Anolis carolinensis*. **J. Morphol. 163:** 331-348.

Courtens, J. C. & Depeiges, A. 1985. Spermiogenesis of *Lacerta vivipara*. **J. Ultrastruct. Res. 90:** 203-220.

Cruz-Landim, C. & Cruz-Höfling, M. A. 1977. Electron microscope study of lizard spermiogenesis in *Tropidurus torquatus* (Lacertilia). **Caryologia 30:** 151-162.

Dekoninck, J. 1991. Biological rhythms associated with sllep and psychological adaptation. **J. Psych. & Neurosc. 16:** 115-122.

Drumond, H. & Burghardt, G. M. 1983. Nocturnal and diurnal nest emergence in green iguanas. **J. Herpetol. 17:** 290-292.

Dugan, B. 1982. A fieldy study of the headbob displays of male green iguanas (*Iguana iguana*): variation in form and context. **An. Behav. 30:** 327-338.

Fawcett, D. W. & Phillips, D. M. 1970. Recent observations on the ultrastructure and development of the mammalian spermatozoon. *In:* Baccetti, B. (edit) **Comparative Spermatology**, p. 13-28. Rome: Accademia Nazionale dei Lincei.

Fawcett, D. W.; Anderson, W. A. & Phillips, D. M. 1971. Morphogenetic factors influencing the shape of the sperm head. **Develop. Biol. 26:** 220-251.

Fitch, H. S. 1940. A field study of the growth and behavior of the fence lizard. **Univ. Calif. Publ. Zool. 44:** 151-172.

Fitch, H. S. 1970. Reproductive cycles of lizards and snakes. **Misc. Publ. 52, Univ. Kansas Mus. Nat. Hist:** 1-127.

Fox, W. & Dessauer, H. C. 1957. Photoperiodic stimulation of appetite and growth in the male lizard, *Anolis carolinensis*. **J. Exp. Zool. 134:** 557-575.

Fox, W. & Dessauer, H. C. 1958. Response of the male reproductive system of lizards (*Anolis carolinensis*) to unnatural day-lengths in different seasons. **Biol. Bull. Mar. Biol. Lab. Wood. Hol. 115:** 421-439.

Gavaud, J. 1991. Role of cryophase temperature and thermophase duration in thermoperiodic regulation of the testicular cycle in the lizard *Lacerta vivipara*. **J. Exp. Zool. 260:** 239-246.

Ghasal, S. K. & Bandyopadhyay, T. K. 1983. Duration of meiosis and spermiogenesis in the common indian garden lizard *Calotes versicolor* (Lacertilia: Lacertidae). **Rev. Brasil. Genét. VI:** 425-432.

Goldberg, S. R. 1970. Seasonal ovarian histology of the ovoviviparous Iguanidae lizard *Sceloporus jarrovi* Cope. **J. Morphol. 132:** 265-276.

Goldberg, S. R., & Lowe, C. R.. 1966. The reproductive cycle of the western whiptail lizard (*Cnemidophorus tigris*) in Southern Arizona. **J. Morphol. 118:** 543-548.

Guillette, L. J., Jr. & Casas-Andreu, G. 1987. The reproductive biology of the high elevation Mexican lizard *Barisia imbricata*. **Herpetologica 43:** 29-38.

Guillette, L. J., Jr. & Sullivan, W. P. 1985. The reproductive and fat body cucles of the lizard, *Sceloporus formosus*. **J. Herpetol. 19:** 474-480.

Guraya, S. S. 1971. Histochemical observations on the lizard spermiogenesis. **Acta Anat. 79:** 270-279.

Hahn, W. E. 1964. Seasonal changes in testicular and epididymal histology and spermatogenic rate in the lizard *Uta stansburiana stejnegeri*. **J. Morphol. 115:** 447-460.

Haldar, C. M. N. & Thaphiyal, J. P. 1980. Inverse seasonal cytological cycles in testes and pineal organs of the bloodsucker *Calotes versicolor*. **Herpetologica 36:** 116-119.

Halpert, A. P.; Garstka, W. R. & Crews, D. 1982. Sperm transport and storage and its relation to the annual sexual cycle of the female red-sided garter snake, *Thamnophis sirtalis parietalis*. **J. Morphol. 174:** 149-159.

Hamilton, D. W. & Fawcett, D. W. 1968. Unusual features of the neck and middle-piece of snake spermatozoa. **J. Ultrastruct. Res. 23:** 81-97.

Healy, J. M. & Jamieson, G. M. 1994. The ultrastructure of spermatogenesis and epididymal spermatozoa of the Tuatara *Sphenodon punctatus* (Sphenodontida: Amniota). **Phil. Trans. R. Soc. Lond. B 344:** 187-199.

Hirth, H. F. 1963. Some aspects of the natural history of *Iguana iguana* on a tropical strand. **Ecology 44:** 613-615.

Jamieson, B. G. M.; Scheltinga, D. M. & Tucker, A. D. 1997. The ultrastructure of spermatozoa of the Australian freshwater crocodile, *Crocodylus johnstoni* Krefft, 1873 (Crocodylidae, Reptilia). **J. Submicr. Cytol. Pathol. 29:** 265-274.

Klein, E. H. 1982. Reproduction of the green iguana (*Iguana iguana* L.) in the tropical dry forest of southern Honduras. **Brenesia 19/20:** 301-310.

Licht, P. 1967a. Enviromental control of annual testicular cycles in the lizard *Anolis carolinensis*. I. Interaction of light and temperature in the initiation of testicular recrudescence. **J. Exp. Zool. 165:** 505-516.

Licht, P. 1967b. Enviromental control of annual testicular cycles in the lizard *Anolis carolinensis*. II. Seasonal variations in the effects of photoperiod and temperature on testicular recrudescence. **J. Exp. Zool. 166:** 243-254.

Licht, P. 1970. Regulation of the annual testis cycle by photoperiod and temperature in the Lizard *Anolis carolinensis*. **Ecology 52:**240-252.

Licht, P. and Gorman, G. C. 1975. Altitudinal effects on the seasonal testis cycles of tropical *Anolis* lizard. **Copeia 3:** 496-504.

Marion, K. R. & Sexton, O. J. 1971. The reproductive cycle of the lizard *Sceloporus malachiticus* in Costa Rica. **Copeia 1971:** 517-526.

Mayhew, W. W. & Wright, S. J. 1970. Seasonal changes in testicular histology of three species of the lizard Genus *Uma*. **J. Morphol. 130:** 163-186.

Newton, W. D. & Trauth, S. E. 1992. Ultrastructure of the spermatozoon of the lizard *Cnemidophorus sexlineatus* (Sauria: Teidae). **Herpetologica 48:** 330-343.

Ortega, A. & Barbault, R.. 1984. Reproductive cycles in the mesquite lizard *Sceloporus grammicus*. **J. Herpetol. 18:** 168-175.

Phillips, D. M. 1972. Comparative analysis of mammalian sperm motility. **J. Cell. Biol. 53:** 561-573.

Pratt, N. C.; Phillips, J. A.; Alberts, A. C. & Boda, K. S. 1994. Functional versus physiological puberty an analysis of sexual bimaturism in the green iguana, *Iguana iguana*. **An. Behav. 47:** 1101-1114.

Ramirez-Pinilla, M. P. 1992. Histological changes in male reproductive tracts of some species of Liolaemus (Reptilia: Sauria: Iguanidae) in different stage of reproductive activity. **Rev. Brasil. Biol. 52:** 133-140.

Rand, A. S. & Bock, B. C. 1992. Size variation, growth and survivorship in nesting green iguanas (*Iguana iguana*) in Panama. **Amphibia-Reptilia 13:** 147-156.

Rand, A. S. & Dugan, B. A. 1980. Iguana egg mortality within the nest. **Copeia 1980:** 531-534.

Reddy, P. R. K. & Prasad, M. R. N. 1970. Hormonal control of the maintenance of spermatogenesis and sexual segment in the Indian house lizard *Hemidactylus flaviviridis* Rüppell. **Gen. And Comp. Endocrin. 14:** 15-24.

Robinson, J. G. & Redford, K. H. 1991. **Neotropical wildlife use and conservation**, p. 181-201. Chicago. The University of Chicago press.

Saita, A.; Comazzi, M. & Perrota, E. 1988. New data at the E. M. on the spermiogenesis of *Iguana delicatissima* (Laurent) involving comparative significance. **Acta Embryol. Morphol. Exper. n. s. 9:** 105-114.

Sanyal, M. K. & Prasad, M. R. N. 1965. Seasonal variation in testis and total body cholesterol in the Indian house lizard, *Hemidactylus flaviviridis* Rüppel. **Steroids 6:** 313-322.

Sanyal, M. K. & Prasad, M. R. N. 1967. Reproductive cycle of the Indian house lizard, *Hemidactylus flaviviridis* Rüppell. **Copeia 1967:** 627-633.

Sprando, R. L. & Russel, L. D. 1988. Spermiogenesis in red-ear turtle (*Pseudemys scripta*) and the domestic fowl (*Gallus domesticus*): a study of cytoplasmic elimination. **J. Morphol. 198:** 95-118.

Srinivas, S. R.; Shivanandappa, T.; Hegde, S. N. & Sarkar, H. B. D. 1995. Sperm storage in the oviduct of the tropical rock lizard, *Psammophilus dorsalis*. **J. Morphol. 224:** 293-301.

Vanzolini, P. E.; Ramos Costa, A. M. M. & Vitt, L. J. 1980. **Répteis da Caatinga.** 1ª Edição. Rio de Janeiro. Academia Brasileira de Ciências.

Wilhoft, D. C. 1963. Gonadal histology and seasonal changes in the tropical Australian lizard, *Leiolopisma rhomboidalis*. **J. Morphol. 113:** 185-203.

Wilhoft, D. C. & Quay, W. B. 1961. Testicular histology and seasonal changes in the Lizard, *Sceloporus occidentalis*. **J. Morphol. 108:** 95-106.

Yasuzumi, G. & Yasuda, M. 1968. Spermatogenesis in animals as revealed by eletron microscopy. XVIII. Fine structure of developing spermatids of the Japanese freshwater turtle fixed with potassium permanganate. **Z. Zellforsch. Mikrosk. Anat. 85:** 18-33.

Young, L. J.; Godwin, J.; Grammer, M.; Gahr, M. & Crews, D. 1995a. Reptilian sex steroid receptors amplification, sequence and expression analysis. **J. Steroid Biochem. Mol. Biol. 55:** 261-269.

Young, L. J.; Nag, P. K. & Crews, D. 1995b. Species differences in estrogen receptor messenger RNA expression in the brain of sexual and unissexual *Whiptail* lizards. **J. Neuroendocrinol. 7:** 567-576.

Zug, G. R. 1993. **Herpetology: An Introductory Biology of Amphibians and Reptiles.** Washington, Academic Press.

6. ARTIGOS CIENTÍFICOS SUBMETIDOS À PUBLICAÇÃO

6.1. **Male Reproductive cycle of *Iguana iguana* (Reptilia: Sauria: Iguanidae) in the Pantanal region, Brazil**

Ferreira *et al.* 2001

Submetido à: Journal of Herpetology

6.2. **Ultrastructural spermiogenesis in the testis of *Iguana iguana* (Reptilia: Sauria: Iguanidae)**

Ferreira and Dolder. 2001

Submetido à: Herpetologica

Authors: FERREIRA *ET AL.*

Running title: REPRODUCTION IN *IGUANA IGUANA*

MALE REPRODUCTIVE CYCLE OF *IGUANA IGUANA* (REPTILIA: SAURIA: IGUANIDAE) IN THE PANTANAL REGION, BRAZIL

ADELINA A. F. FERREIRA,[1,3] IRACELES A. LAURA,[2] AND HEIDI DOLDER[1]

[1] *Departamento de Biologia Celular, IB, Unicamp, Campinas, São Paulo, Brazil*

[2] *Departamento de Morfofisiologia, CCBS, UFMS, Campo Grande, Mato Grosso do Sul, Brazil*

[3] To whom correspondence should be addressed: *Departamento de Biologia Celular, IB, C.P. 6109, Unicamp, Campinas 13083-970, São Paulo, Brazil.* adelinaf@unicamp.br

ABSTRACT

Adult specimens (N= 10) of the lizard *Iguana iguana* were collected the different seasons in the Brazilian Pantanal. Their testes were removed and processed by conventional techniques for light and scanning eletron microscopy. During the annual reproductive cycle, an inversely proportional relationship was observed in the development of seminiferous tubules versus interstitial tissue, thus characterizing four stages, which alternate between a maximal peak and a minimal low point of spermatogenesis. The first stage (October-December) is the low point of spermatogenesis, with only the initial divisions of spermatogonia, while the interstitial tissue and Leydig cells are extremely evident. This condition continues during the second stage (January-March) a resting period and reduction of the interstitial tissue. The third stage (April-June) is the maximal peak of germ cell activity with all degrees of cell maturation present while the interstitial tissue is reduced. In the last season (July-September) spermiation has already occurred, with large amounts of residual cytoplasm still in the seminiferous tubule lumen. The possible responses verified for this cycle in relation to group behavior and seasonal ecological alterations.

The reproductive cycle of tropical lizards show great variability (Van Sluys, 1993). The variation of testis development, morphologically established, is conditioned to seasonal periodicity, which is an ecologically important characteristic (Fitch, 1970). Species of temperate zones have been described as to their reproductive cycle by De Wolte and Telford (1966); Sanyal and Prasad (1967); Kasinathan and Basu (1973); Schrank and Ballinger (1973); Gavaud (1991).

Morphological variations are characteristic of the testes at different times in the annual reproductive cycle of lizards. The developmental stages of germ cells are observed concomitantly as to the variation in quantity and metabolic activity of the interstitial tissue (Fox and Dessauer, 1958; Wilhoft and Quay, 1961; Wilhoft, 1963; Hahn, 1964; Del Conte, 1972), so that these can be inversely proportional (Goldberg and Lowe, 1966).

There are several factors that determine this cycle, both intrinsic and extrinsic to the lizard, but the preponderant factor in the *I. iguana* seems to be climatic variation (photoperiod, temperature and precipitation), which appears to induce their reproductive period (This was also verified for other lizards by Fox and Dessauer, 1958; Hahn, 1964; Licht, 1967a; 1967b; 1970; Mayhew and Wright, 1970; Licht and Gorman, 1975).

The behavior of both sexes during the reproductive cycle of *I. iguana* demonstrates their adaptability to conditions essential for reproductive success in temperate regions (Dugan, 1982; Klein, 1982; Rodda, 1991; 1992; Pratt *et al.*, 1994). For Hirth (1963), *I. iguana* is a lizard that usually reproduces in conditions of higher temperatures. However, according to Rand (1968) and Fitch (1970) this feature can be modified, depending on climatic conditions of the region where this species lives.

I. iguana (Lineu, 1748) is a large green herbivorous lizard, about 1.6 meters long, with an arboreal habitat, that inhabits the neotropics. In Brazil, it is found in the Amazon Basin, along the Cerrado Rivers, and in the Pantanal region (Robinson and RedFord, 1991).

Based on this knowledge, the present work describes the annual reproductive cycle of *I. iguana* males, using structural and ultrastructural characteristics of the testes, and considering the seasonal variations in the Brazilian Pantanal. This study is a contribution

toward the understanding of testis morphology of *I. iguana*, during their annual reproductive cycle. Little is known about this process in tropical, regularly flooded areas such as the Brazilian Pantanal. Our data may contribute to ecological and behavioral studies.

MATERIALS AND METHODS

Study area.— Adult specimens of *I. iguana* (N = 10) were collected in the "Pantanal" region of the state of Mato Grosso do Sul, Central Brazil (see Figure 1), during the period of September 1998 to July 2000. The Pantanal is the largest regularly flooded area in the center of South America, with approximately 168,000 km^2, between 14°-22°S and 53°-61°W (Carvalho, 1984), and is composed of diverse systems strongly conditioned by seasonal floods (Brown, 1984). The land slopes very gently, which hinders rainwater drainage (Prance and Schaller, 1982). The climate is of the tropical, sub humid type with annual temperatures oscillating between 23 and 42° C. The annual rainfall reaches an average of 1,500mm (Alvarenga *et al.*, 1984) with alternation of the rainy period from October to March, and the dry from April to September. So that, based on the flood cycle, we have four seasons: the Flood ("Cheia"), Drainage ("Vazante"), Drought ("Seca") and Inundation ("Enchente") (Adamoli, 1984).

Light microscopy.— The specimens were anesthetized by ethyl ether inhalation and first measured for biometry data (see Table 1). The testes were individually removed and measured. They were fixed for 12 hours in Bouin, Carnoy or Alfac (80% alcohol, 15% formol and 5% acetic acid) solutions, cut in half, and returned for another 6 hours in the fixatives. After dehydration in a graded ethanol series, they were embedded in paraffin. Sections were cut at 5 μm and stained with hematoxylin-eosin.

Scanning eletron microscopy.— Bouin's fixed testes were also washed in 0.1M cacodylate buffer for two days, then infiltrated in progressively more concentrated sucrose solutions, up to 3%. After fracturing in liquid nitrogen, they were post fixed in 1% osmium tetroxide and dehydrated in an ethanol series. They were critical point dried, sputter coated with gold, and observed with a Jeol JSM-5800LV Scanning Electron Microscope.

RESULTS

Male reproductive system. — The organs of the male reproductive system of *I. iguana* are located dorso-longitudinally in the abdominal cavity (Fig. 2A). The rounded testes are connected to the epididymis and vas deferens, which contact the kidneys, posteriorly in the body cavity. These organs were histologically identified. The vas deferens ducts reach the copulating organ, which is inserted in the tail base, and everted during copulation.

Testis morphology. — The testis of I. iguana are enveloped by a mesothelium and a tunica albuginea, but the seminiferous tubules are not arranged in parallel within the testes (Figs. 2B-2C). The seminiferous tubules are surrounded by a layer of myoid cells and germ cell (Figs. 3A and 3D). Inside the tubules, the germinal epithelium is made up of two types of cells, the nutritive or Sertoli cells and the germ cells (Figs. 2D, 3A-3D). The different cell stages in the germinal epithelium are found depending on the phase of testicular development. Within the seminiferous tubule, the germ cells are distributed from the periphery to the central lumen, according to their degree of maturity (Figs. 3A-3D and 4A-4D). The seminiferous tubules are sustained by interstitial tissue, made up of a loose connective tissue, rich in veins, nerves and Leydig cells (Figs. 3A, 4C, 4D). These cells are large, numerous, round or polygonal, with granular cytoplasm, and are usually arranged in groups.

Four stages of the annual spermatogenic cycle can be distinguished (Figs. 3A-3D), which correspond to the four annual seasons. Two peaks were observed (Figs. 4A-4D). In stage 1, December, the initial division of germinal cells begins. Spermatogonia are abundant and primary spermatocytes make up the intervening layers to the tubule lumen. The interstitial tissue and Leydig cells are quite evident (Fig. 3A and 4A, 4C), making this the peak of interstitial tissue activity. March (stage 2) is a resting period. The testicular structure is similar to the previous period, although the interstitial tissue, specially the Leydig cells diminish, while spermatogenic cells begin to divide (Fig. 3B). A maximum peak of spermatogenetic activity marks June (stage 3). Germ cells in all maturation stages are present and numerous sperm are in the lumen. The interstitial tissue is almost absent (Fig. 3C and 4B, 4D). In September (stage 4), soon after the copulation, mature germ cells

are absent. Some primary spermatocytes and spermatogonia are still present. Large amounts of residual cytoplasm are observed in the lumen (Fig. 3D). The graph (Fig. 5) concisely shows the correlation of seasonal changes in the Pantanal (involving temperature and precipitation) with the testis dimentions.

DISCUSSION

The division, proliferation and maturation of germinal cells, follow a cycle with a short maximum peak, and long periods of stability, resulting in a characteristic annual testicular cycle in *I. iguana*. This is similar to the process previously described for lizards. However, most authors affirm that the maximum spermatogenetic peak occurs in Summer for the lizards of temperate climates (Goldberg, 1970; Ballinger and Nietfeldt, 1989; Gavaud, 1991). In these lizards, the reproductive cycle has a peak of spermatogenic activity in conditions of high temperatures and long phototoperiods (Goldberg and Lowe, 1966; Sanyal and Prasad, 1967; Kasinathan and Basu, 1973).

For some authors, spermatogenesis and spermiation is only triggered by exposure to long photoperiods, occurring in natural conditions, during the long summer days (Fox and Dessauer, 1958; Licht, 1967a), always associated with high temperatures, usually above 30°C (Hahn,1964; Mayhew and Wright, 1970; Gavaud, 1991). These conditions are necessary to stimulate germ cell proliferation and spermiation. This has been demonstrated in experimental conditions, for the lizard *Anolis carolinensis*, (Licht, 1967b; 1970), which inhabits the tropics. This species in experimental conditions has a long reproductive period, from March to August (Licht and Gormam, 1975).

Other researchers believe in the possible association of some extrinsic factors, such as diet conditions, hormonal factors (Wilhoft and Quay, 1961), the amount of rain (De Wolfe and Telford, 1966) or variations in altitude (Licht and Gorman, 1975) to determine the reproductive cycle. Lizards, such as the Australian *Leiolopisma rhomboidalis* (Wilhoft, 1963), have a slower process of spermatogenesis, extending throughout the year, but spermiation occurred only in Spring. Another lizard with continuous spermiogenesis was *Cnemidophorus lemniscatus* (Del Conte, 1972).

Tropical lizards present a noticeable adaptive capacity to different climatic conditions; thus these lizards present different needs for reproduction and development (Marion and Sexton, 1971; Licht and Gorman, 1975; Ortega and Barbault, 1984; Guillette and Sullivan, 1985; Guillette and Casas-Andreu, 1987; Ramirez-Pinilla, 1991). For the genus *Sceloporus*, inhabitants of different tropical areas, the cycle is quite variable, with the reproductive period usually occurring in Summer (Sanyal and Prasad, 1967; Goldberg, 1970; Ortega and Barbault, 1984; Méndez-de la Cruz *et al.*, 1988; Guillette and Méndez-de la Cruz, 1993). Similar patterns were found for *Liolaemus huacahuasicus* in Argentina (Ramirez-Pinilla, 1991) and *Barisia imbricata* in the mountains of Mexico (Guillette and Casas-Andreu, 1987). However, *Sceloporus formosus* in Mexico reproduced in Spring (Guillette and Sullivan, 1985), while for *Sceloporus malachiticus* from Costa Rica, the cycle was found to occur in Summer (Marion and Sexton, 1971). Thus it is clear that the reproductive cycle of the different species in tropical countries is very homogeneous if we consider the high temperature and the longer photoperiod as the most important environmental factors. Precipitation can also be an important fator (Guillette and Casas-Andreu, 1987).

The *I. iguana* is known to adjust efficiently to environmental conditions, resulting in variations of several corporal characteristics, as was observed by some authors (Fitch, 1970; Trajano and Ghiringuello, 1978). We also observed such adaptions. The body size and weight were not directly related to variations in testis size during the cycle, contrary to the descriptions of the above authors. However, testis data are directly related to variations in climate, as it can be observed in Table 1. However the microscopical variations (Figures 3 and 4) demonstrate these modifications even more clearly.

Some authors found a relation between adaptive behavior and atmospheric conditions for *I. iguana* (Hirth, 1963; Dugan, 1982; Klein, 1982; Pratt et al., 1994). The reproductive process must be adapted to environmental needs to guarantee the successful development of embryo and the young lizard (Rand, 1968; Fitch, 1970). However, there is little information relating the testicular microscopic morphology of *I. iguana* to environment, and few places present the environmental conditions, with periodic floods as in the Brazilian Pantanal. Some speculations can be made on the reproductive process of male green iguana in the Brazilian Pantanal. This cycle seems to be the result of a series of

physiological solutions to adapt this group to its environmental conditions. This arrangement permits the meeting of couples, followed by mating, oviposition and appropriate conditions for embryonic development (Hirth, 1963; Fitch, 1970; Klein, 1982). The environment, characterized by the hot flood season and cold drought, only happens in this area, which makes comparisons difficult.

The maximum peak of spermatogenic production occurred in the Pantanal drainage season (June), characterized by low temperatures and a short photoperiod. However the few rains and the drying out of the forests bordering the rivers now permit the meeting and formation of couples for copulation. This would have been impossible during the flood period (March), even though the high temperatures and longer photoperiod are usually considered favorable for lizard reproduction (field observations).

The spermiation period in male *I. iguana*, in reality precedes the reproductive cycle of iguana females by about six to seven months, permitting egg development inside the ovary after copulation. The probable storage of sperm in follicles should be investigated. The laying of eggs, about 30 on the average, is carried out in holes in sun exposed sand, distant from the forests, since high temperatures are necessary for embryo development. The birth period, about 7 to 8 months after copulation, also must have high temperatures to guarantee the survival of the young lizards which are highly sensitive to temperature variations. The food supply is much larger in the flood period. Neonatal iguanas are bright green and do not yet present the adult's ability to change color according to their environment. Therefore the environment with many green plants also protects them from predators. The dislocated reproductive cycle of the males provides favorable seasonal conditions for the development of embryos and the young (field observations). Fitch (1970) alsi observed this, in part.

Some authors showed that the interstitial tissue also presents an annual cycle (Fox and Dessauer; Wilhoft and Quay, 1961; Wilhoft, 1963; Hahn, 1964). The Leydig cells have been observed to increase cyclically in volume and activity (Goldberg and Lowe, 1966; Del Conte, 1972).

Thus, it is very clear that the iguana's reproductive activity is influenced by external factors (also noted by Rand and Bock, 1992), being strongly adaptable to environmental conditions (Fitch, 1970). Hormonal variation can influence the annual reproductive cycle in

lizards (Reddy and Prasad, 1970). However, this variation is influenced by atmospheric conditions, temperature and photoperiod (Bartholomew, 1950; Fox and Dessauer, 1957; Licht, 1967a; 1967b; Duvall et al., 1982). In tropical areas, the increase or reduction of precipitation is particularly important (Guillette and Sullivan, 1985; Guillette and Casas-Andreu, 1987; Méndez-de la Cruz, 1988; Guillette and Méndez-de la Cruz, 1993).

The reproductive cycle of *I. Iguana* is annual, and the period of maximum testis development is relatively short, occuring in the months of July to the September. It is important to mention that pertinent literature shows that lizards (exactly in tropical regions) typically have their reproductive period in the summer. The high temperatures were considered the stimulating factor of spermatogenesis. On the other hand, for *I. Iguana*, the seasonal drought and flood seasons stimulated and adaptation to the period that permis the formation of couples for copulation followed by a warmer period adequate for egg and embryonic development.

The correlation of testis morphology during the reproductive cycle, taking in consideration behavior and the relationships of several lizard groups, as well as the morphology of the female reproductive tract, deserves further attention.

Acknowledgments. — We thank A. V. L. Freitas for reviewing the manuscript; L. A. Viana for his help in the field work; the IBAMA that granted authorization for the collection of a few Pantanal specimens, since these animals are protected by Brazilian law. We are grateful to the Coordinators for permitting the use of the Pantanal Research Base, of the Universidade Federal do Mato Grosso do Sul and to C. Strussmann for the climatic data. This study was suported by FAPESP (Proc. 00/03259-5).

TABLE 1

Environmental conditions of the Brazilian Pantanal during the period studied. Macroscopic biometry of *I. iguana* (body wet mass and snout-vent length = SVL) and testes (length and width).

Period	N	Body wet mass (g)	SVL (cm)	Testes (cm)
September /1998 (Drought)	2	500	28	2.0x0.5
		1500	45	2.3x1.5
December /1998 (Inundation)	2	1000	33	2.0x0.5
		2500	41	2.4x1.0
March /1999 (Flood)	1	500	33	0.5x0.8
June /1999 (Drainage)	2	2000	40	3.0x2.0
		2000	39	2.5x2.0
October /1999 (Inundation)	2	2500	41	1.4x0.9
		1800	36	1.5x0.9
July /2000 (Drought)	1	2315	38	2.8x1.3

FIGURE LEGENDS

Figure 1: Map locating the study area. **A.** Arrow indicates the Brazilian Pantanal, on the South American continent. **B.** Detailed map of this region, bordered by the principal rivers.

Figure 2: **A.** Male reproductive system of *I. iguana*. The testes (T) have reached their maximum size during the reproductive period. Epididymis (E). The vas deferens (D) are intimately associated with the kidneys (K). The copulating organ is inserted in the tail (curved arrow). Natural size. **B.** Testis section observed with light microscopy, shows tangential sections of the seminiferous tubules, confirming their irregular arrangement (arrows). X64. **C.** Seminiferous tubules observed with SEM, are twisted in different directions (arrows). Capsule that covers the testis (arrow head). X320. **D.** Seminiferous tubule, under the SEM, where the germinal epithelium, during the reproductive period, is organized in columns. It is possible to observe spermatogonia (G), spermatocytes (C) and spermatozoa (Z) regions. X800.

Figure 3: Transverse sections of the testis in different stages of spermatogenesis. **A.** Stage 1, with spermatogonia (G) and primary spermatocytes (P) reaching to the lumen; interstitial tissue (IT) is abundant and Leydig cells (L) are large. **B.** Stage 2, the germ cells initiate proliferation while the interstitial tissue (IT) begins to diminish. Notice the spermatogonia (G), primary spermatocytes (P), secondary spermatocytes (S) and a few spermatids (T). **C.** Stage 3 with all stages of germ cells present. Spermatozoa (Z) are abundant near the lumen. Interstitial tissue (IT) is almost absent. **D.** In stage 4, mature cells have been eliminated, leaving a great amount of residual cytoplasm (R). Only spermatogonia (G) are observed. Interstitial tissue (IT) is developing. Hematoxylin-Eosin staining. X600.

Figure 4: SEM micrographs. **A.** Lumen of a seminiferous tubule (L) in stage 1, with spermatogenic cells in reduced proliferation and some spermatocytes (C) reaching the lumen (L). Interstitial tissue (IT) surrounds the tubule (arrows). X1600. **B.** Lumen of a seminiferous tubule in stage 3 with a well developed germ cell layer. (Z - spermatozoa).

X1600. **C.** Interstitial tissue (IT) of testis in stage 1. Fibrous tissue increases (arrow) as the seminiferous tubules' diameter (ST) is reduced. X5400. **D.** Interstitial tissue (IT) of testis in stage 3. Is reduced (arrows) surrounding the developed seminiferous tubules (ST). X5400. Figures **C.** and **D.** Bars indicate the different thicknesses of the interstitial tissue (IT).

Figure 5: Graph concisely showing the seasonal variation in testis size (stars) correlated to climatic changes, i.e. temperature (lines) and precipitation (vertical bars) in the Pantanal. Modified from Tarifa *et al.*(1986). These data are relatively constant from year to year.


Brasil
A

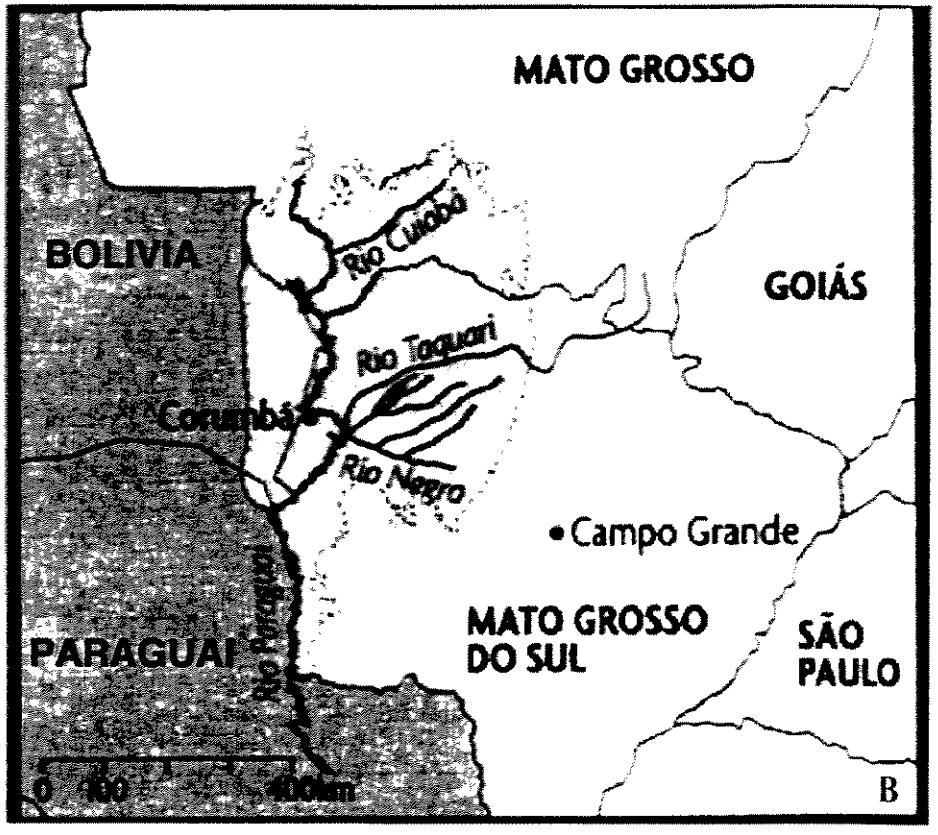
MATO GROSSO
BOLIVIA
Rio Cuiabá
GOIÁS
Rio Taquari
Corumbá
Rio Negro
Campo Grande
MATO GROSSO
DO SUL
SÃO
PAULO
PARAGUAI
Rio Paraguai
0 100 400km
B

Figure 1

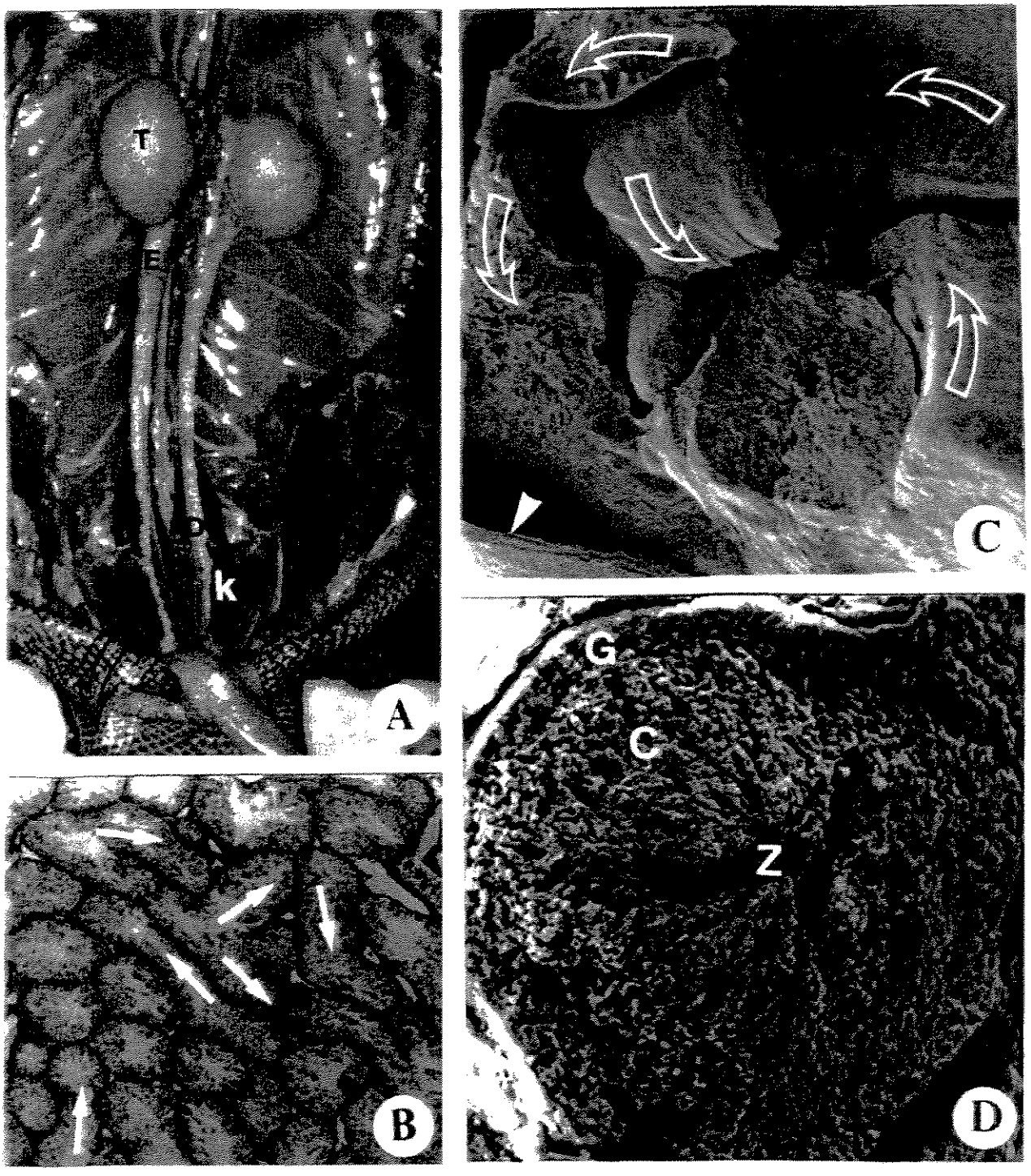

Figure 2

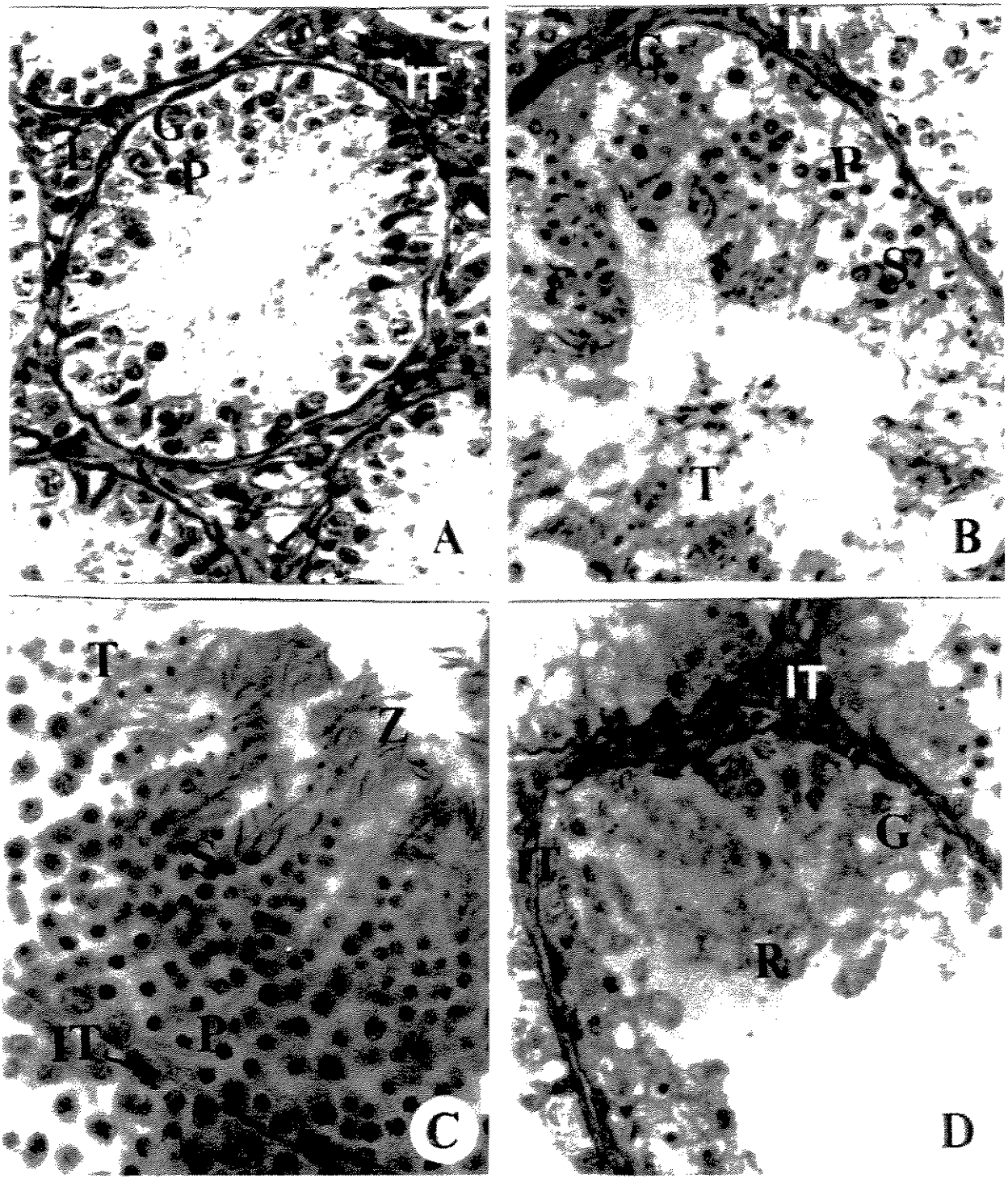

Figure 3

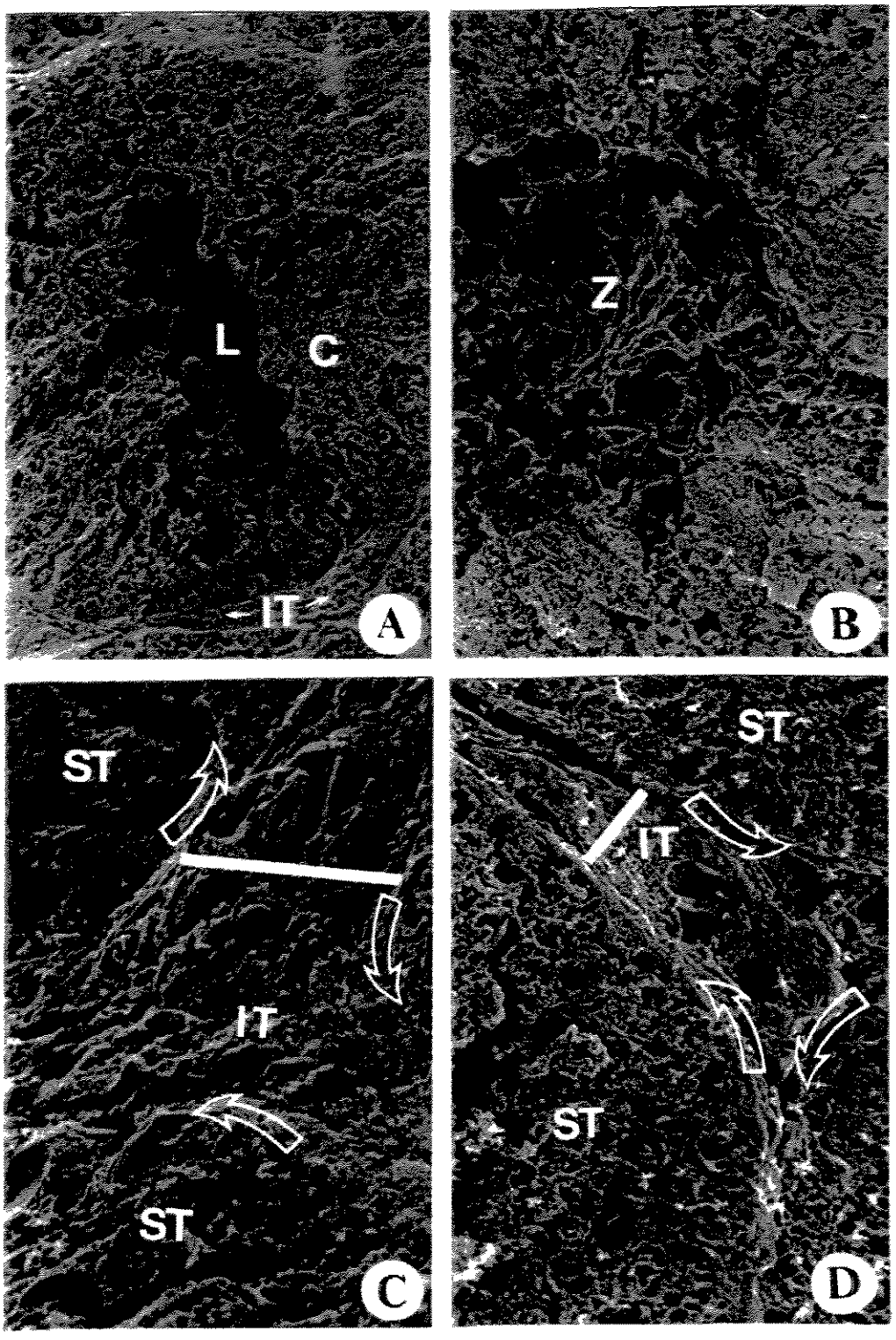

Figure 4

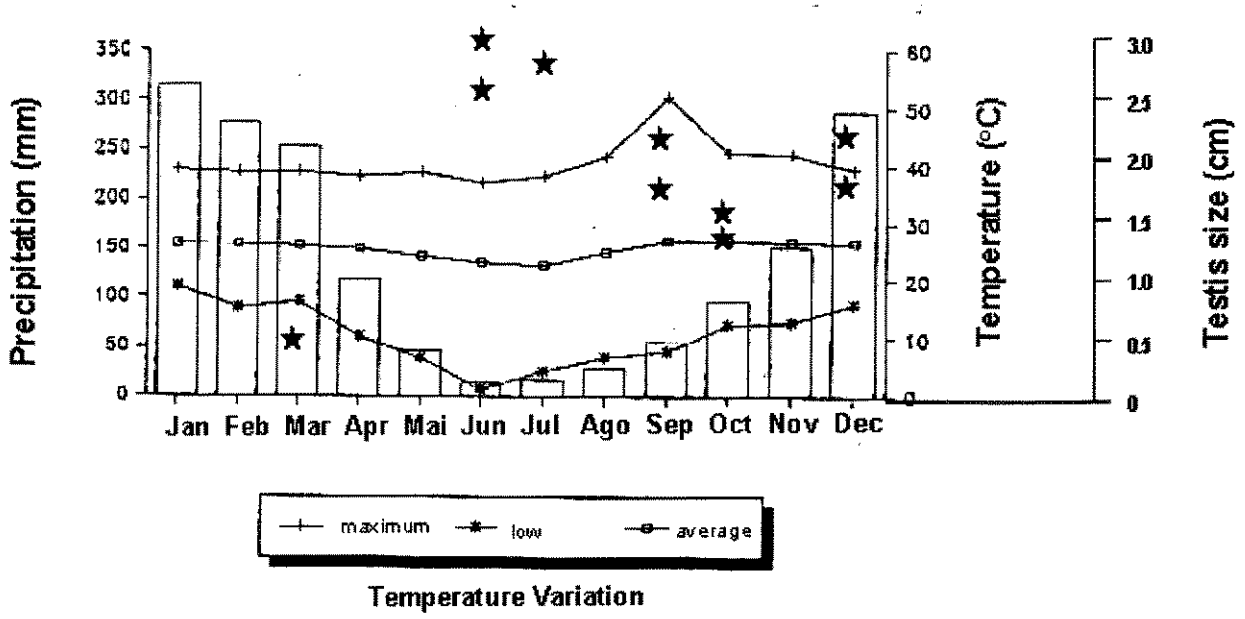
Precipitation (mm)
350
300
250
200
150
100
50
0
Jan Feb Mar Apr Mai Jun Jul Ago Sep Oct Nov Dec
60
50
40
30
20
10
0
Temperature (°C)
30
25
20
15
10
0.5
0
Testis size (cm)
maximum
low
average
Temperature Variation

Figure 5

LITERATURE CITED

ADÁMOLI, J. 1984. A dinâmica das inundações no Pantanal. In: Simpósio sobre Recursos Naturais e Sócio Econômicos do Pantanal: 51-61. Embrapa-Pantanal. Corumbá, MS.

ALVARENGA, S. M., A. E. BRASIL, R. PINHEIRO AND H. J. H. KUX. 1984. Estudo geormorfológico aplicado à bacia do Alto Rio Paraguai e Pantanais Mato-Grossenses. Bol. Tec. Proj. Radam Brasil 187: 89-183.

BALLINGER, R. E. AND J. W. NIETFELDT. 1989. Ontogenetic stages of reproductive maturity in the viviparous lizard, *Sceloporus jarrovi* (Iguanidae). J. Herpetol. 23: 282-292.

BARTHOLOMEW, G. A. 1950. The effects of artificially controlled temperature and day length on gonadal development in a lizard, *Xantusia vigilis*. Anat. Rec. 106: 49-59.

BROWN, Jr., K. S. 1984. Zoogeografia da Região do Pantanal Mato Grossense. In: Simpósio sobre Recursos Naturais e Sócio Econômicos do Pantanal: 137-178. Embrapa-Pantanal. Corumbá, MS.

CARVALHO, N. O. 1984. Hidrologia da Bacia do Alto Paraguai. In: Simpósio sobre Recursos Naturais e Sócio Econômicos do Pantanal: 43-49. Embrapa-Pantanal. Corumbá, MS.

CHILDRESS, J. R. 1970. Observations on the reproductive cycle of *Agama stellio picea*. Herpetologica 26: 149-155.

DEL CONTE, E. 1972. Variación cíclica del tejido intersticial del testículo y espermiogénesis continua em un lagarto Teído tropical: *Cnemidophorus l. leminiscatus* (L.). Act. Cient. Venez. 23: 177-183.

DE WOLTE, B. B., AND S. R. TELFORD. 1966. Lipid-positive cells in the testis of the lizard, *Cnemidophorus tigris*. Copeia 1966: 590-592.

DUGAN, B. 1982. A field study of the headbob displays of male green iguanas (*Iguana iguana*): variation in form and context. An. Behav. 30: 327-338.

DUVALL, D., L. J. GUILLETTE, Jr., AND R. E. JONES. 1982. Enviromental control of reptilian reproductive cycles. *In* C. Gans and H. Pough (eds.), Biology of the Reptilia. Pp. 201-231. Vol. 13, Academic Press, New York.

FITCH, H. S. 1970. Reproductive cycles of lizards and snakes. Misc. Publ. 52, Univ. Kansas Mus. Nat. Hist: 1-127.

FOX, W., AND H. C. DESSAUER. 1957. Photoperiodic stimulation of appetite and growth in the male lizard, *Anolis carolinensis*. J. Exp. Zool. 134: 557-575.

FOX, W., AND H. C. DESSAUER. 1958. Response of the male reproductive system of lizards (*Anolis carolinensis*) to unnatural day-lengths in different seasons. Biol. Bull. Mar. Biol. Lab. Wood. Hol. 115: 421-439.

GAVAUD, J. 1991. Role of cryophase temperature and thermophase duration in thermoperiodic regulation of the testicular cycle in the lizard *Lacerta vivipara*. J. Exp. Zool. 260: 239-246.

GOLDBERG, S. R., AND C. R. LOWE. 1966. The reproductive cycle of the Western whiptail lizard (*Cnemidophorus tigris*) in Southern Arizona. J. Morphol. 118: 543-548.

_____ 1970. Seasonal ovarian histology of the ovoviviparous Iguanidae lizard *Sceloporus jarrovi* Cope. J. Morphol. 132: 265-276.

GUILLETTE, L. J., Jr., AND F. R. MENDEZ-DE LA CRUZ. 1993. The reproductive cycle of the viviparous Mexican lizard *Sceloporus torquatus*. J. Herpetol. 27: 168-174.

_____ AND W. P. SULLIVAN. 1985. The reproductive and fat body cycles of the lizard, *Sceloporus formosus*. J. Herpetol. 19: 474-480.

_____ AND G. CASAS-ANDREU. 1987. The reproductive biology of the high elevation Mexican lizard *Barisia imbricata*. Herpetologica 43: 29-38.

HAHN, W. E. 1964. Seasonal changes in testicular and epididymal histology and spermatogenic rate in the lizard *Uta stansburiana stejnegeri*. J. Morphol. 115: 447-460.

HIRTH, H. F. 1963. Some aspects of the natural history of *Iguana iguana* on a tropical strand. Ecology 44: 613-615.

KASINATHAN, S., AND S. L. BASU. 1973. Seasonal variation in the testes, acessory ducts and adrenal glands of *Calotes versicolor* (Daudin). Act. Morphol. Acad. Sci. Hung. 21 (3): 271-279.

KLEIN, E. H. 1982. Reproduction of the green iguana (*Iguana iguana* L.) in the tropical dry forest of southern Honduras. Brenesia 19/20: 301-310.

LICHT, P. 1967a. Enviromental control of annual testicular cycles in the lizard *Anolis carolinensis*. I. Interaction of light and temperature in the initiation of testicular recrudescence. J. Exp. Zool. 165: 505-516.

_____ 1967b. Enviromental control of annual testicular cycles in the lizard *Anolis carolinensis*. II. Seasonal variations in the effects of photoperiod and temperature on testicular recrudescence. J. Exp. Zool. 166: 243-254.

_____ 1970. Regulation of the annual testis cycle by photoperiod and temperature in the Lizard *Anolis carolinensis*. Ecology 52:240-252.

_____ AND GORMAN, G. C. 1975. Altitudinal effects on the seasonal testis cycles of tropical *Anolis* lizard. Copeia 1975: 496-504.

MARION, K. R. AND O. J. SEXTON. 1971. The reroductive cycle of the lizard *Sceloporus malachiticus* in Costa Rica. Copeia 1971: 517-526.

MAYHEW, W. W., AND S. J. WRIGHT. 1970. Seasonal changes in testicular histology of three species of the lizard Genus *Uma*. J. Morphol. 130: 163-186.

MÉNDEZ- DE LA CRUZ, F. R., L. J. Jr. GUILLETTE, M. V. SANTA CRUZ, AND G. CASAS-ANDREW. 1988. Reproductive and fat body cycles of the viviparous lizard *Sceloporus mucronatus* (Sauria: Iguanidae). J. Herpetol. 22:1-12.

ORTEGA, A., AND R. BARBAULT. 1984. Reproductive cycles in the mesquite lizard *Sceloporus grammicus*. J. Herpetol. 18: 168-175.

PRANCE, G. T., AND G. B. SCHALLER. 1982. Preliminar study of some vegetation types of the Pantanal, Mato Grosso, Brazil. Brittonia 34: 228-251.

PRATT, N. C., J. A. PHILLIPS, A. C. ALBERTS, AND K. S. BODA. 1994. Functional versus physiological puberty an analysis of sexual bimaturism in the green iguana, *Iguana iguana*. An. Behav. 47: 1101-1114.

RAMIREZ-PINILLA, M. P. 1991. Reproductive and fat body cycles of the viviparous lizard *Liolaemus huacahuasicus*. J. Herpetol. 25: 205-208.

RAND, A. S. AND B. C. BOCK. 1992. Size variation, growth and survivorship in nesting green iguanas (*Iguana iguana*) in Panama. Amphibia-Reptilia 13: 147-156.

REDDY, P. R. K., AND M. R. N. PRASAD. 1970. Hormonal control of the maintenance of spermatogenesis and sexual segment in the Indian house lizard *Hemidactylus flaviviridis* Rüppell. Gen. Comp. Endocrinol. 14: 15-24.

RODDA, G. H. 1991. Sexing *Iguana iguana*. Bull. Chicago Herpetol. Soc. 26: 173-175.

_____ 1992. The mating behavior of *Iguana iguana*. Smithson. Contrib. Zool. 534: 1-40.

SANYAL, M. K., AND , M. R. N. PRASAD. 1967. Reproductive cycle of the Indian house lizard, *Hemidactylus flaviviridis* Rüppell. Copeia 1967: 627-633.

_____ 1965. Seasonal variation in testis and total body cholesterol in the Indian house lizard, *Hemidactylus flaviviridis* Rüppel. Steroids 6: 313-322.

SCHRANK, G. D., AND R. E. BALLINGER. 1973. Male reproductive cycles in two species of lizards (*Cophosaurus texanus* and *Cnemidophorus gularis*). Herpetologica 29: 289-293.

TRAJANO, E., AND A. GHIRINGHELLO. 1978. Comparação entre as proporções corporais de *Iguana iguana* da região das Caatingas e da Amazônia (Sauria: Iguanidae). Pap. Av. Zool. 32: 107-115.

VAN SLUYS, M. 1993. The reproductive cycle of *Tropidurus itambere* (Sauria: Tropiduridae) in Southeastern Brazil. J. Herpetol. 27: 28-32.

WILHOFT, D. C. 1963. Gonadal histology and seasonal changes in the tropical Australian lizard, *Leiolopisma rhomboidalis*. J. Morphol. 113: 185-203.

_____ AND W. B. QUAY. 1961. Testicular histology and seasonal changes in the lizard, *Sceloporus occidentalis*. J. Morphol. 108: 95-106.

Authors: FERREIRA AND DOLDER

ULTRASTRUCTURAL SPERMIOGENESIS IN THE TESTIS OF *IGUANA IGUANA* (REPTILIA: SAURIA: IGUANIDAE)

ADELINA A. F. FERREIRA AND HEIDI DOLDER

Departamento de Biologia Celular, IB, C.P. 6109, Unicamp, Campinas 13083-970, São Paulo, Brazil. adelinaf@unicamp.br

ABSTRACT

Iguana iguana spermiogenesis was studied by transmission and scanning electron microscopy. During this process, grouped structures such as the acrosomal complex in the spermatid head and the axoneme complex in the middle and principal pieces of the flagellum are formed. The nuclear content is compacted initially into thick, longitudinal chromatin filaments. Nuclear shape is determined by further compaction and by the manchette, a layer of microtubules surrounding the head. The acrosomal complex originates from Golgi vesicles and the interaction between the pre-acrosomal vesicle and the nucleus. The middle piece presents a centriolar pair, surrounded by a fibrous sheath and rings of simple and modified mitochondria. The centrioles sustain the axoneme that appears at the end of the middle piece. It extends throughout the principal piece of the flagellum with the 9 + 2 pattern, still surrounded by the fibrous sheath. In the end piece, the axoneme continues, surrounded only by the plasma membrane. In the seminiferous tubules' lumen, the spermatozoa still retain plentiful residual cytoplasm, which classifies them as immature at the moment of spermiation.

Key words: Spermiogenesis, testis, electron microscopy, *Iguana iguana*, Reptilia, Sauria.

Spermiogenesis is the process of biochemical and structural modifications of spermatids that results in the production of spermatozoa (Fawcett *et al.*, 1971). The spermatozoon is usually a small, highly compact cell specialized in ovum fertilization (Clark, 1967). Several authors describe spermiogenesis in Lacertilia (Guraya, 1971; Cruz Landim and Cruz Höfling, 1977; Courtens and Depeiges, 1985; Saita *et al.*, 1988; Al-Dokhi, 1997), many with emphasis on the head formation (Clark, 1967; Butler and Gabri, 1984; Al-Hajj *et al.*, 1987; Cruz Höfling and Cruz Landim, 1978) and the spermatozoon ultrastructure of Squamata (Furieri, 1970; Newton and Trauth, 1992a; Healy and Jamieson, 1992; Oliver *et al.*, 1996; Jamieson *et al.*, 1996). Some specially focus on the Sertoli cells (Dufaure, 1971; Saita *et al.*, 1988). Other studies use the spermatozoan morphology as an instrument for phylogeny (Jamieson and Healy, 1992; Healy and Jamieson, 1994; Harding *et al.*, 1995; Teixeira *et al.*, 1999a; 1999b). The final spermatozoon maturation related to the reproductive cycle in the reproductive tract of lizards has been described (Fox, 1963; Cuellar, 1966; Conner and Crews, 1980; Adams and Cooper, 1988; Carcupino *et al.*, 1989; Newton and Trauth, 1992b). This research was undertaken to describe the process as a whole and detail some ultra-structural aspects of intra-testis spermiogenesis of *Iguana iguana*.

MATERIAL AND METHODS

Male specimens of *I. iguana* were collected in the Pantanal (14°-22°S and 53°-61°W) of Mato Grosso do Sul, Brazil, in the center of South America. The animals were anesthetized by ethyl ether inhalation; the testes removed and fixed overnight with solutions containing 4% glutaraldehyde, 4% paraformaldeyde in 0.1M cacodylate buffer (pH 7.2), others fragments were fixed in 4% glutaraldehyde and 1% tannic acid in 0.1M

phosphate buffer (pH 7.2) with 1,8% sucrose. They were post-fixed with 1% osmium tetroxide in the same buffer at 4°C, dehydrated in acetone and embedded in Epon 812 resin. The sections were stained in uranyl acetate and lead citrate and observed with a Zeiss LEO 906 Transmission Electron Microscope (TEM). Other fixed testis fragments were washed in 0.1M cacodylate buffer for two days, then infiltrated in progressively more concentrated sucrose solutions, up to 3%. After fracturing in liquid nitrogen, they were post fixed in 1-% osmium tetroxide and dehydrated in an ethanol series. They were critical point dried, sputter coated with gold, and observed with a Jeol JSM-5800LV Scanning Electron Microscope (SEM).

RESULTS

Several events, during the modification of spermatids, are sequential. The initial spermatids are larger and round, with a irregular surface (Figs. 1A, 1B). When cellular prolongation begins the flagellum is already formed (Figs. 1C).

Many small vesicles (Fig. 1D) originating from the Golgi complex and endoplasmic reticulum are scattered in the cytoplasm of the primary spermatocyte. Later, some of these vesicles fuse into a larger vesicle, lateral to the nucleus (Fig. 1E). Gradual modification of its size and content forms, a clear pro acrosomic vesicle equal in size to the nucleus (Fig. 1F). This vesicle persists with its initial characteristics during a good part of the elongation process, surrounding part of the nucleus (Figs. 1G, 1H). The acrosomal complex is located over the initial portion of the nucleus of spermatids (Fig. 1H, 1I, 2B, 3A). It is made up of the acrosome and the perforatorium (Fig. 3A, 3C). The acrosome in late spermatids presents an acrosomic vesicle constituted by a narrow, clear cortex, and a wide, homogeneous, electron dense core (Fig. 3A) covering the sub acrosomic cone (Figs. 2A, 3A, 3B, 3C). Below the sub acrosomic cone, is a narrow layer known as the epinuclear zone, fill by an eletron transparent substance (Figs. 3A, 3B, 3C). Inside the pro acrosomic vesicle, an electron dense granule occurs, which will later form the perforatorium (Fig. 1F). This granule elongates into the area between the vesicular and nuclear membranes, undergoing modifications (Figs. 2A, 3A, 3C). The perforatorium in early spermatids is

located between the clear epinuclear zone and the nucleus (Fig. 2A, 3A, 3B). It becomes rod-like filled with fibrous material (Figs. 3A, 3C), and its elongation occurs at the same time as that of the nucleus and acrosome (Figs. 1G, 1H, 2A, 3A).

The nucleus in early spermatids is large and central, with filaments of fine chromatin, dispersed or in clots (Figs. 1E). In advanced spermatids the chromatin is organized in dense fibers, which appear initially in a helical arrangement (Fig. 1G, 1I, 1J), and later extend longitudinally (Fig. 1H). They become progressively thicker and more tightly packed, finally forming a long, completely dense nucleus (Figs. 2A, 2B, 2C, 2D). The nucleus in the immature spermatozoa is cylindrical and slightly curved (Fig. 2B, 2C, 2E). The acrosome is conical, surrounding the anterior nuclear portion and accompanying its curvature (Figs. 2B, 3A).

In the central portion of the nucleus of late spermatids and immature spermatozoa, a transition can be found between the gouged anterior portion and the lower cylindrical nucleus, which has been called the nuclear shoulders, and marks the limit of the acrosomal complex (Figs. 1H, 2A, 2B, 3A). Longitudinal and cross sections of immature spermatozoa show many layers that surround the nucleus, including the acrosomal complex, the spermatid's plasma membrane and the Sertoli cell's plasma membrane (Figs. 3A, 3B, 3C). The Sertoli cell emits cytoplasmic process throughout the seminiferous tubules, covering the head area of the spermatids and immature spermatozoa (Fig. 3E, 3F).

The microtubules are helically arranged around the nucleus in early spermatids, while in advanced spermatids the microtubules are longitudinally disposed (Figs. 2A, 2B, 2C, 3C, 3D), forming a structure known as the "manchette". This structure is associated with the spermatids embedded in the Sertoli cell (Fig. 3C, 3E, 3F), surrounding the acrosome and nucleus (Figs. 1J, 2C, 3C, 3D), lying parallel to the chromatin fibers during nuclear compaction (Figs. 1J, 2C) and to the acrosomic complex in formation (Figs. 1H, 1I, 2A, 3C).

Early spermatids present an invagination in the nuclear base, in which the middle piece is implanted, containing the two centrioles at the flagellar base (Figs. 2C, 2D, 4E). A typical proximal centriole is central and perpendicular to the nucleus, while the distal one extends parallel to the nucleus (Figs. 4A, 4B, 4C). Microtubules extend from this centriole forming the axoneme (Fig. 4A, 4B, 4F, 4G, 4H, 4I). Still in the neck region, the first dense

bodies are being formed by the deposition of electron dense material in some mitochondria (Fig. 4C, 4F).

During the elongation of the spermatids several mitochondria move to the nuclear base (Fig. 2D, 4A) and are modified, becoming larger, with transverse cristae (Figs. 4A, 4B, 4F, 4G). The middle piece or neck area is made up of mitochondria in circular arrays around the initial portion of the axoneme, alternating with dense bodies, that are mitochondria modified at the end of spermiogenesis (Figs. 4C, 4F). The mitochondria are organized around the distal centriole, above the fibrous sheath (Figs. 4B, 4F, 4G). The transition of the middle piece to the principal piece is marked by the annulus, an electron dense fold of the plasma membrane (Figs. 4B, 4F). The flagellum is long and cylindrical (Figs. 1C, 3E, 4B, 4D, 4E), mostly made up of the principal piece, which consists in the axoneme surrounded by peripheral dense fibers, a fibrous sheath, cytoplasm and the plasma membrane (Figs. 4B, 4F, 4H, 4I). The axoneme is composed of associated microtubules in a 9 + 2 pattern, of nine doublets surrounding a pair of single microtubules. Each doublet in the middle piece is linked to a peripheral dense fiber (Figs. 4F, 4G), specially well developed for the doublets 3 and 8, which are linked to the annulus and the fibrous sheath below it (Fig. 4F, 4G). These fibers are not observed in the flagellum, after the initial portion of the axoneme (Fig. 4H, 4I). These are surrounded by the fibrous sheath, seen in cross section as regular, separate rings (Fig. 4B, 4H). The end piece is the narrowest area of the flagellum (Fig. 4J), consisting only in the axoneme, with its typical pattern and the plasma membrane.

The spermatozoa are liberated in the lumen of the seminiferous tubule still with a certain amount of cytoplasm, particularly around the head (Fig. 4K). Large mitochondria can be found in the middle piece, and the dense bodies are not fully differentiated (Figs. 4C, 4F).

DISCUSSION

During the spermiogenesis of *I. iguana*, several cytoplasmic and nuclear changes can be seen. Some are common to the several species and others only to Lacertilia. The

spermatids can be classified in several types (Courtens and Depeiges, 1985; Healy and Jamieson, 1994). However, it is important to observe that sequences of events exist, several of them simultaneous, so that it is difficult to establish the exact beginning and ending of each stage. The acrosome is an organelle rich in enzymes, which permits the entrance of the spermatozoon into the ovum (Baccetti and Afzellius, 1976). In most Squamata this organelle presents a wide variation of sizes, forms and layers (Furieri, 1970). In lizards, the acrosome is long and curved, with an acrosomal vesicle and the typical sub-acrosomal cone (Fawcett *et al.*, 1971; Baccetti *et al.*, 1980). The acrosome can be homogeneous and electron dense (Furieri, 1974; Cruz Landim and Cruz Höfling, 1977; Cruz Höfling and Cruz Landim, 1978) or in *I. iguana* and other species with a clear cortex and an electron dense core (Al-Hajj *et al.*, 1987; Jamieson, 1995; Oliver *et al.*, 1996). The sub-acrosomal cone is a structure only found in Squamata (Jamieson and Scheltinga, 1993). The epinuclear zone is evident in most of the spermatozoa of Lacertilia (Jamieson *et al.*, 1996; Oliver *et al.*, 1996).

The early spermatids of *I. iguana* present a slightly dense area between the sub-acrosomal cone and the acrosome, similar to that found in *Tropidurus semitaeniatus*, *T. torquatus* (Teixeira *et al.*, 1999a) and in *Amphisbaena alba* (Teixeira *et al.*, 1999b). The perforatorium in terrestrial animals is formed by filamentous actin, which permits its extraordinary extension capacity (Baccetti and Afzellius, 1976; Baccetti *et al.*, 1980). This organelle, according to some authors, acts only as a support for the acrosome (Baccetti *et al.*, 1980), but it is more probable to have a mechanical function during ovum penetration and during the acrosome reaction (Shiroya *et al.*, 1986). During the extension process the filaments are rearranged from a helical to a parallel arrangement. This happens due to the sliding of actin filaments (Baccetti, 1979; Shiroya *et al.*, 1986). The origin of the perforatorium during the spermiogenesis is not known and no enzymatic activity has been detected in it (Guraya, 1971). It is a common structure in spermatozoa of non-passeriformes birds and all the Squamata (Sprando and Russel, 1988). Their origin from the electron dense granule inside the early pro acrosomic vesicle has been described (Clark, 1967; Cruz Landim and Cruz Höfling, 1978; Saita *et al.*, 1988) as also happens in birds (Nagano, 1962; Humphreys, 1975). The granule seems to appear at the point of contact between nucleus and pro acrosomic vesicle (Del Conte, 1976; Courtens and Depeiges, 1985), or by the

association of actin filaments in this contacting area (Al-Hajj *et al.*, 1987). In lizards the perforatorium is anterior to the nucleus, or it can be located covering the nuclear tip (Butler and Gabri, 1984; Courtens and Depeiges, 1985; Saita *et al.*, 1988; Healy and Jamieson, 1994). However in Sphenodontia (Healy and Jamieson, 1992; 1994; Jamieson and Healy, 1992), Crocodilia (Saita *et al.*, 1987) and Chelonia (Sprando and Russel, 1988) the perforatorium is inside a nuclear channel.

The principal characteristic of the spermatozoon nucleus in Lacertilia is the extreme compacting of the nuclear material and its long, thin format (Bergstrom and Arnold, 1974; Jamieson *et al.*, 1996; Oliver *et al.*, 1996; Teixeira *et al.*, 1999a). This shape favors mobility and the compacting protects the genome from physical and chemical influences during the storage and transport to the ovum (Krause, 1996).

Nuclear shape is established and maintained during spermiogenesis, by the aggregation pattern of the chromatin (Fawcett *et al.*, 1971) and by the appearance of a structure called "manchette", composed of microtubules associated around the nucleus (Clark, 1967; Myles and Hepler, 1977; Cruz Höfling and Cruz Landim, 1978; Courtens and Depeiges, 1985; Al-Dokhi, 1997). The manchette is a common structure during spermiogenesis of all the Lacertilia and can have an helical formation (Al-Hajj *et al.*, 1987), longitudinal (Courtens and Depeiges, 1985; Saita *et al.*, 1988; Al-Dokhi, 1997) or initially circular, becoming longitudinal (Clark, 1967; Cruz Landim and Cruz Höfling, 1977; Cruz Höfling and Cruz Landim, 1978; Butler and Gabri, 1984), as was also found for *I. iguana* spermiogenesis.

At the transition point, between the anterior portion to the main part of the nucleus, there is a tapered region called the nuclear shoulders, and it is very evident in *I. iguana*. It occurs in several lizards (Cruz Landim and Cruz Höfling, 1977; Cruz Höfling and Cruz Landim, 1978; Butler and Gabri, 1984; Jamieson *et al.*, 1996; Oliver *et al.*, 1996; Teixeira *et al.*, 1999a). It is discreet in *Anolis carolinensis* (Clark, 1967) and *Cnemidophorus sexlineatus* (Newton and Trauth, 1992a; 1992b), and observed with difficulty in *Agama stellio* (Al-Hajj, 1987) and *Sphenodon punctatus* (Healy and Jamieson, 1992).

The neck area is short in most Squamata, where the centrioles are involved in the formation of the axonemic complex (Furieri, 1970). In Lacertilia the distal centriole is shorter than in Chelonia and Sphenodontia (Jamieson and Healy, 1992; Healy and

Jamieson, 1994; Jamieson, 1995; Jamieson *et al.*, 1996; Oliver *et al.*, 1996). The function of the distal centriole can be compared to one of the basal corpuscles at the axoneme base, where it acts as a mold for the pattern of the flagellum microtubules (Fawcett and Phillips, 1970). The distal centriole does not exist in mammals (Fawcett, 1970; Phillips, 1972), but it is common in Anura (Furieri, 1975; Amaral *et al.*, 1999) and in birds (Asa and Phillips, 1987; Asa *et al.*, 1986). In some Squamata, birds and mammals, a laminate structure is observed close to the proximal centriole, resulting from the polymerization of molecular subunits (Phillips and Olson, 1975; Jamieson *et al.*, 1996; Oliver *et al.*, 1996) and observed in *I. iguana*.

In most animals, the middle piece is quite long (Phillips and Asa, 1993), however, in Squamata it is small or moderately long (Hamilton and Fawcett, 1968; Jamieson, 1995; Jamieson *et al.*, 1996; Oliver *et al.*, 1996) as was observed in early *I. iguana* spermatids. The total number of mitochondria (Phillips and Asa, 1993) determines the length of the middle piece. The mitochondria of early spermatids in Squamata are rounded (Cruz Landim and Cruz Höfling, 1977; Saita *et al.*, 1988; Newton and Trauth, 1992a), column shaped (Al-Dokhi, 1997), tubular and sinuous (Furieri, 1970; Jamieson *et al.*, 1996; Teixeira *et al.*, 1999a) or between round and columnar as in spermatids of *I. Iguana* and other Lacertilia (Courtens and Depeiges, 1985; Oliver *et al.*, 1996). In all lizards, transverse sections of the middle piece show mitochondria forming either complete rings around the axoneme (Courtens and Depeiges, 1985; Oliver *et al.*, 1996) or, in the case of Squamata, incomplete rings interrupted by dense bodies, as also occurs in *I. iguana* (Furieri, 1970; Healy and Jamieson, 1992; Jamieson *et al.*, 1996; Oliver *et al.*, 1996; Al-Dokhi, 1997; Teixeira *et al.*, 1999b). It has been suggested that dense bodies are formed by mitochondrial modification (Carcupino *et al.*, 1989; Healy and Jamieson, 1992; Jamieson and Healy, 1992). In *I. iguana* the formation of the dense bodies is one of the last events of spermiogenesis. In Squamata there are dense bodies forming complete rings (Jamieson *et al.*, 1996), longitudinal arrays (Furieri, 1970), in a spiral (Oliver *et al.*, 1996) or irregularly dispersed, as was observed in *I. iguana*, (Harding *et al.*, 1995, Teixeira *et al.*, 1999a). The function of the dense bodies is not known.

In *I. iguana*, the slightly dense areas found between the mitochondria are interpreted as being dense bodies in formation. The total modification of the mitochondria of *I. iguana*

appears only to happen after the spermatozoon has left the testis. Perhaps energy is necessary for the immature spermatozoon to leave of the testis and arrive in the spermatic ducts and all the mitochondria initially need to be active. After final maturation of the spermatozoon, some mitochondria differentiate into dense bodies, in order to reduce the size of the middle piece and consequently of the spermatozoon as a whole. In Sphenodontia the dense bodies are small and located in the center of the mitochondria (Healy and Jamieson, 1992; Jamieson and Healy, 1992). As in all the Squamata, the mitochondria of *I. iguana* spermatids have transverse cristae (Guraya, 1971; Cruz Landim and Cruz Höfling, 1977; Courtens and Depeiges, 1985; Saita *et al.*, 1988; Al-Dokhi, 1997). However, in Sphenodontia, Crocodilia and Chelonia (Yasuzumi and Yasuda, 1968; Furieri, 1970; Phillips, 1970; Saita *et al.*, 1987; Healy and Jamieson, 1992; 1994; Jamieson and Healy, 1992) the mitochondria present transverse cristae only during initial spermiogenesis, acquiring cristae in a concentric arrangement, with a dense central body in early spermatids. According to Yasuzumi and Yasuda (1968) the concentric cristae in Sphenodontia provide an increased surface area, and possibly have appeared in response to conditions demanding great mitocondrial activity. For Healy and Jamieson (1992) the spermatozoon of birds, mammals and other Squamata developed during evolution, some form of cristae modification at the end of spermiogenesis.

The annulus is a common structure in Squamata (Jamieson *et al.*, 1996; Oliver *et al.*, 1996; Al-Dokhi, 1997; Teixeira *et al.*, 1999a) and in many invertebrates (Baccetti and Afzellius, 1976). This structure, formed by a group of intimately associated filamentous units (Furieri, 1970) seems to impede the displacement of mitochondria from the middle piece along the flagellum during the movement (Fawcett, 1970).

The 9+2 structure of the axoneme is typical of most animals and all Reptiles (Jamieson *et al.*, 1997). The peripheral dense fibers originate by protein deposition and a motor function associated to that of the axoneme has been attributed to this structure.(Hamilton and Fawcett, 1968; Phillips, 1972; Phillips and Olson, 1975). In most of the lizards a connection of the two opposing fibers onto the fibrous sheath is observed along the whole axonemic complex (Jamieson *et al.*, 1997; Teixeira *et al.*, 1999a; Teixeira *et al.*, 1999b), but in *I. iguana* this connection is observed only in the middle piece.

The fibrous sheath is originated by the accumulation of an amorphous layer between the axonemic complex and the plasma membrane during spermiogenesis (Fawcett and Phillips, 1970; Soley, 1994). The sheath has elastic properties, which may contribute towards flagellar mobility (Fawcett, 1970). For most vertebrates, this sheath appears only below the flagellar middle piece (Jamieson, 1995). However, in some Lacertilia the fibrous sheath is formed starting from the middle piece (Newton and Trauth, 1992b; Al-Dokhi, 1997), as also happens in *I. iguana*. In the immature spermatozoa of *I. iguana*, the axonemic complex at the end of the middle piece still maintains a connection between the 4 and 8 doublets of the axoneme with the fibrous sheath, as has been commonly observed in lizards (Teixeira *et al.*, 1999a; 1999b; Jamieson *et al.*, 1996; Oliver *et al.*, 1996). However, in the rest of the flagellum this connection does not exist and a considerable space is observed between the axoneme and the fibrous sheath. The flagellum end piece is reduced in diameter, with the end of the fibrous sheath, while the same microtubule pattern remains, as in other Lacertilia (Jamieson *et al.*, 1996; Oliver *et al.*, 1996; Teixeira *et al.*, 1999a).

During spermatid differentiation, these cells are embedded in the Sertoli cell cytoplasm; thus this cell gives support, nutrition, protection and the hormonal supply during all spermiogenesis (Butler and Gabri, 1984; Courtens and Depeiges, 1985; Al-Hajj *et al.*, 1987; Saita *et al.*, 1988). All the structures common to a spermatozoon of Lacertilia (Furieri, 1970; Healy and Jamieson, 1992; Jamieson and Healy, 1992; Jamieson and Scheltinga, 1996; Oliver *et al.*, 1996; Teixeira *et al.*, 1999b) are formed during the spermiogenesis of *I. iguana*. However we observed a large amount of cytoplasm around luminal spermatozoa in the seminiferous tubules. As a result, we can call these "immature" spermatozoa. Structural modifications will probably occur during their passage through the reproductive tract, although this is held to be a rare event in Vertebrates. Spermiogenesis completed in the reproductive tract has been verified in other Squamata (Conner and Crews, 1980; Bouresli *et al.*, 1981; Halpert *et al.*, 1982; Adams and Cooper, 1988; Srinivas *et al.*, 1995). These post-testis modifications have not been located along the reproductive tract, and they possibly are not essential for greater spermatozoon mobility.

In conclusion, the process of spermiogenesis of *I. Iguana* involves a series of ultrastructural modifications that culminate in the production of organelles essencial to the spermatozoon. In general, free spermatozoa are considered to have completed all their

necessary morphological features. However for this species, the spermatozoa liberated by spermiation are not mature. This conclusion was based on the fact that the dense bodies in the middle piece are not yet eletron dense and that cytoplasm is still abundant around the spermatozoon head.

Acknowledgments. — We thank the IBAMA for authorization granted for the collection of a few Pantanal lizard specimens, since Brazilian law protects these animals. This study was supported by FAPESP (Proc. 00/03259-5).

FIGURE LEGENDS

Figure 1: A. B. and **C.** SEM micrographs. **A.** Seminiferous tubule with developing germ cells (s) and the thick wall that covers the tubule (arrow) X1800. **B.** Rounded early spermatids (ES), and elongated spermatids (LS) with a flagellum (f). X6300. **C.** Late spermatids (LS) free in the seminiferous tubule lumen, with a long flagellum (f). X5950. **Figure D. - J.** TEM micrographs. **D.** Spermatocyte with cytoplasm rich in Golgian vesicles (v). The nucleus presents chromatin in clots (*) and the initial chromosome attachment to the synaptonemic complex (arrow), typical of meiosis. X7500. **E.** Initial spermatid, with the formation of two vesicles (v), that will contribute to the acrosome, associated with the nucleus (N). Observe the accumulation of substances at the nuclear - cytoplasm border (arrows). X8400. **F.** Early spermatid, with a electron dense granule (g) inside the pro acrosomal vesicle (v). In the area between vesicle and nucleus, notice an electron dense area (arrow) which possible originates the sub acrosomal cone. X7500. **G.** Spermatid during nuclear elongation: the chromatin begin to condense at the nuclear apex (a), forming thick and helically arranged filaments (arrow). The nuclear tip is now surrounded by the pro acrosomic vesicle (v). X32300. **H.** Spermatid in final chromatin condensation into thick, longitudinal filaments (arrow). The nuclear shoulders appear (NS), and at the nuclear apex, the sub-acrosomal cone development (c) is concomitant to the acrosome (a), still covered by the pro acrosomic vesicle (v). X18000. Figures **I.** and **J.** Transverse sections of the areas marked in figure H. **I.** Apical section of the nucleus (N): notice the thick chromatin filaments, encircled by the sub-acrosomal cone (c), lucid zone (z), pro acrosomic vesicle (V) and plasma membrane of the spermatid (MS). X50000. **J.** Medial section of the nucleus (N) where the manchette is observed (mt) following the same helical arrangement as the chromatin filaments (arrows). X33500.

Figure 2: A. B. C. and **D.** TEM micrographs. Longitudinal sections of a late spermatid head. **A.** Observe the nucleus (N) with highly compacted chromatin. Initial formation of the acrosomal complex, that begins to show the perforatorium (p), epinuclear zone lucid (e), sub-acrosomal cone (c) surrounded by the clear zone (z) and the acrosome (a) at the apex

cell. Around the whole cell, is the manchette (mt). X13950. **B.** In this image it is possible to have an idea of the nuclear length (N), the nuclear shoulder location (NS) and the acrosomal complex, consisting in the sub-acrosomal cone (c) and the acrosome (a). X83600. **C.** The nucleus (N) is surrounded by the manchette (mt) along its entire length. At the nuclear base is the implantation fossa of the middle piece (IF). X12000. **D.** Cytoplasmatic extensions (arrows), with mitochondria (m) arranged around the middle piece and the implantation fossa (IF). X10800. **E.** SEM micrographs. Late spermatids (Ls) and flagellum (f). The broken line outlines the closer spermatid. X5400.

Figure 3: TEM micrographs. **A.** Longitudinal section of the spermatozoon. Nucleus (N), nuclear shoulders (NS) and acrosomal complex with several layers: perforatorium (p), epinuclear zone (e), sub-acrosomal cone (c), clear zone (z), acrosome (a) constituted by the narrow, clear cortex, and a homogeneous, electron dense core. The whole cell is covered by the plasmic membrane spermatozoa (MS) and immersed in the Sertoli cell (SC). X25000. **B. C.** and **D.** Transverse sections of the different areas indicate in figure A. **B.** Mid acrosomal complex. X64700 **C.** Base of the acrosomal complex. X50000. From the external layers toward the center can be identified: Sertoli cell (SC), plasmic membrane of the spermatozoon (MS), microtubules (mt), acrosome (a), clear zone (z), sub acrosomal cone (c), epinuclear zone (e), perforatorium (p) and the nucleus (N). **D.** Nucleus (N) surrounded longitudinally by the manchette (mt) and the Sertoli cell (SC). X38800. **E.** SEM micrograph. Late spermatid (Ls) with the head embedded in the Sertoli cell (SC) and the flagellum (f) free in the lumen. X7200. **F.** Section near the seminiferous tubule lumen (L) where the abundant cytoplasm of several late spermatids (Ls) is embedded in prolongation of the Sertoli cells (SC). X2300.

Figure 4: A. Differentiation of the middle piece (MP) where mitochondria (arrows) are arranged around the distal centriole. X13950. **B.** Longitudinal section of a late spermatid. Middle piece formed by the proximal (PC) and distal centrioles (DC) surrounded by mitochondria in rings (m). Transition area of the middle piece to the flagellum is marked by

the annulus (AN). Making up the flagellum is the axoneme (AX) encircled by rings of the fibrous sheath (FS). X15500. **C.** Late spermatid with nucleus (N), and middle piece including the proximal (PC) and distal centrioles (DC), and showing initial dense bodies (arrows) as a modification alternating among the mitochondrial rings (m). X23300. **D.** and **E.** SEM micrographs. **D.** Spermatozoon, where the head (h), middle piece (mp) and flagellum (f) are observed. X11500. **E.** Criofracture where the head of the spermatozoon was removed, showing the flagellum (f) and implantation fossa (IF). X4500. **F.** and **G.** Transverse sections of different areas of the middle piece indicated in figure B. **F.** Observe the axoneme (AX), the peripheral dense fibers (PF) and the annulus (AN), the mitochondrial ring (m) with a dense body (arrow) in differentiation. X43100. **G.** The axoneme (AX) is surrounded by a ring of fibrous sheath (FS) and another of mitochondria (m). Notice the contact of the larger dense fibers 3 and 8 with the fibrous sheath. X41750. **H.** Axonemic complex that forms the flagellum, composed of the axoneme (AX) and rings of the fibrous sheath (FS), covered by the spermatozoon's plasmic membrane (MS) X30000. **I.** Transverse section of the principal piece of the flagellum with the axonemic complex. Notice the leak of dense fibers associated with the doublets. X30000. **J.** Transverse section of the flagellum's end piece; the axoneme (AX) maintains its (9+2) pattern, but is no longer encircled by fibrous sheath rings. X72000. **K.** Spermatozoa (*) in the lumen of the seminiferous tubule (L). Notice the great amount of cytoplasm around the nucleus (*) and free flagella (arrows). X3000.

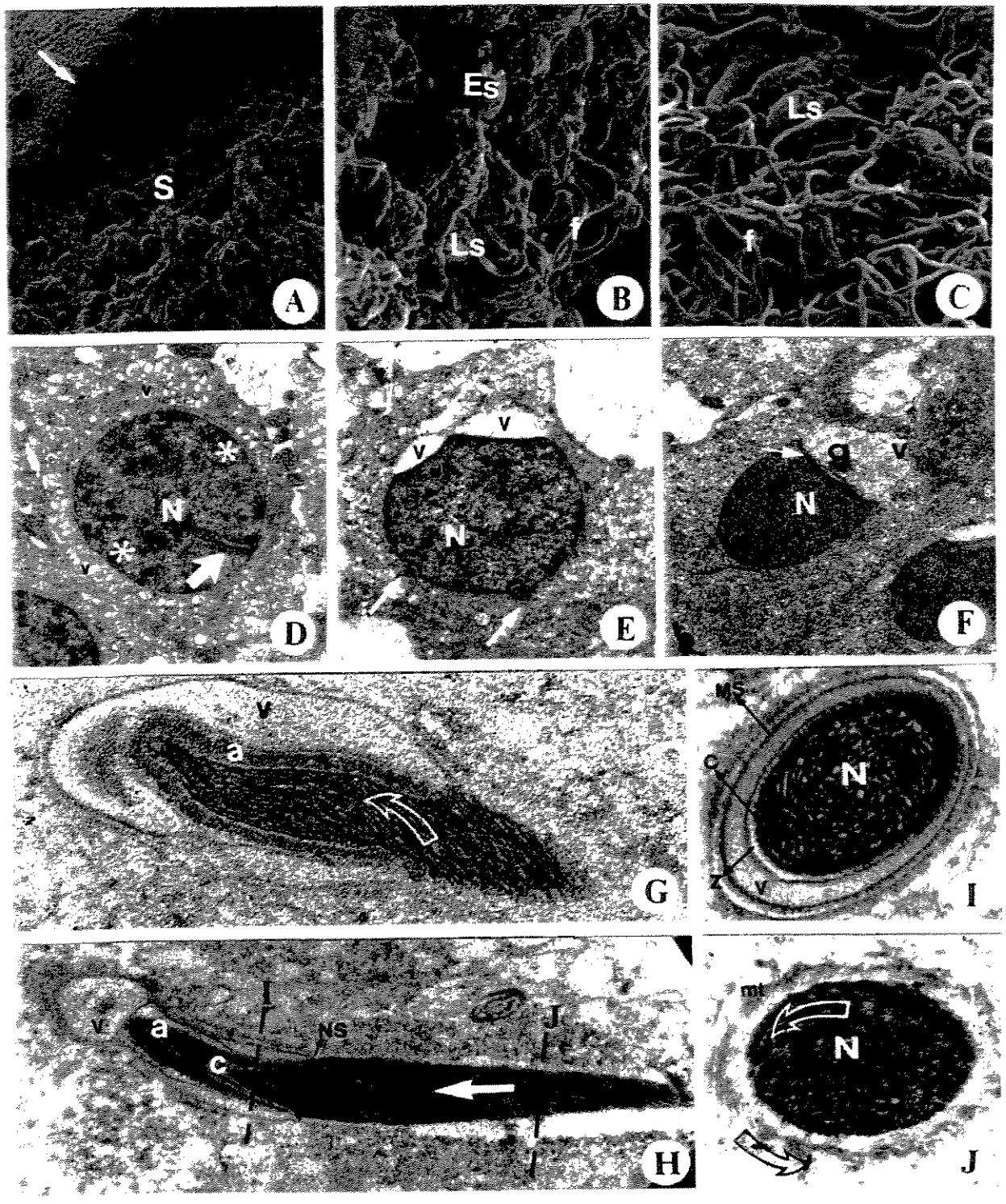

Figure 1

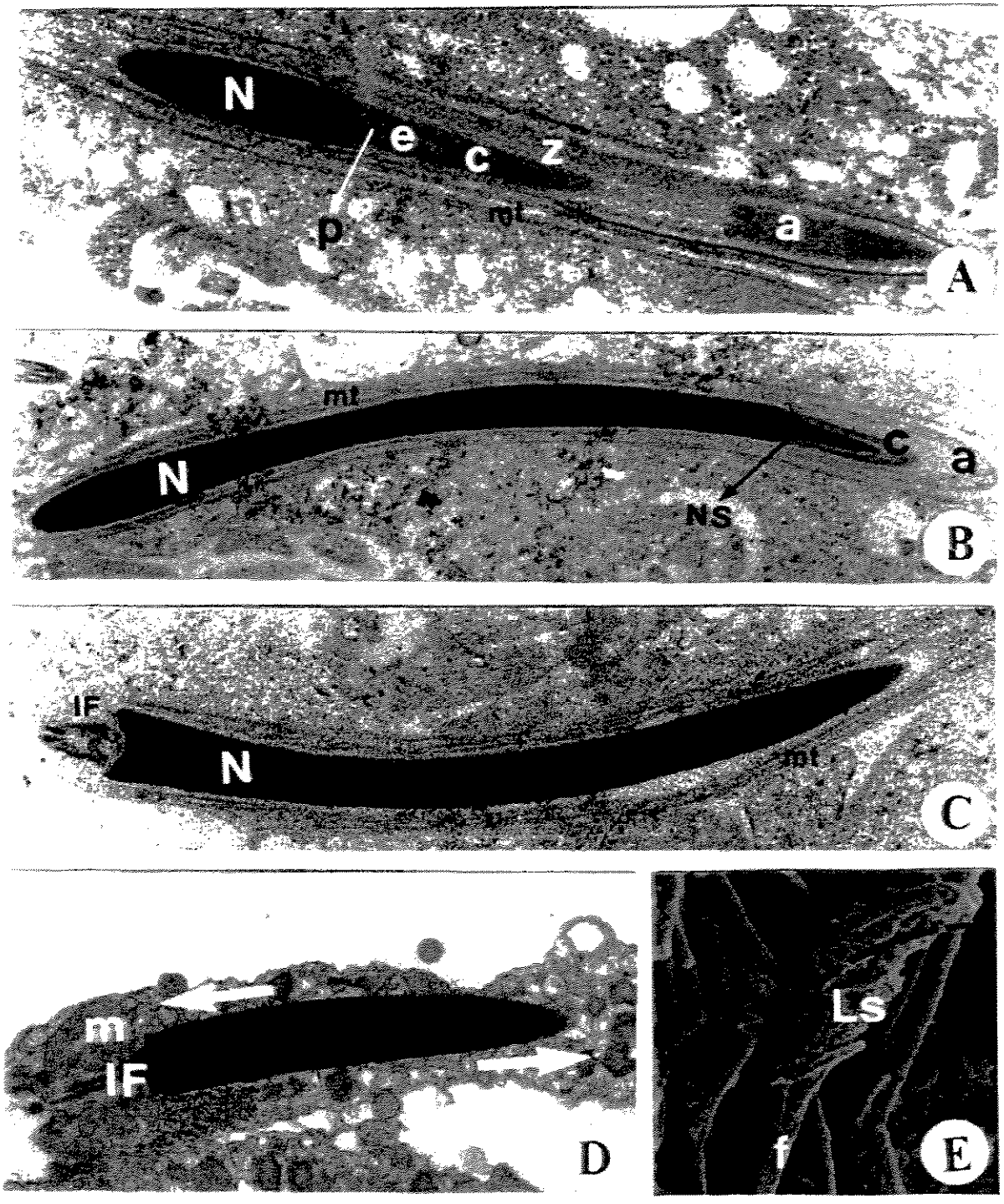

Figure 2

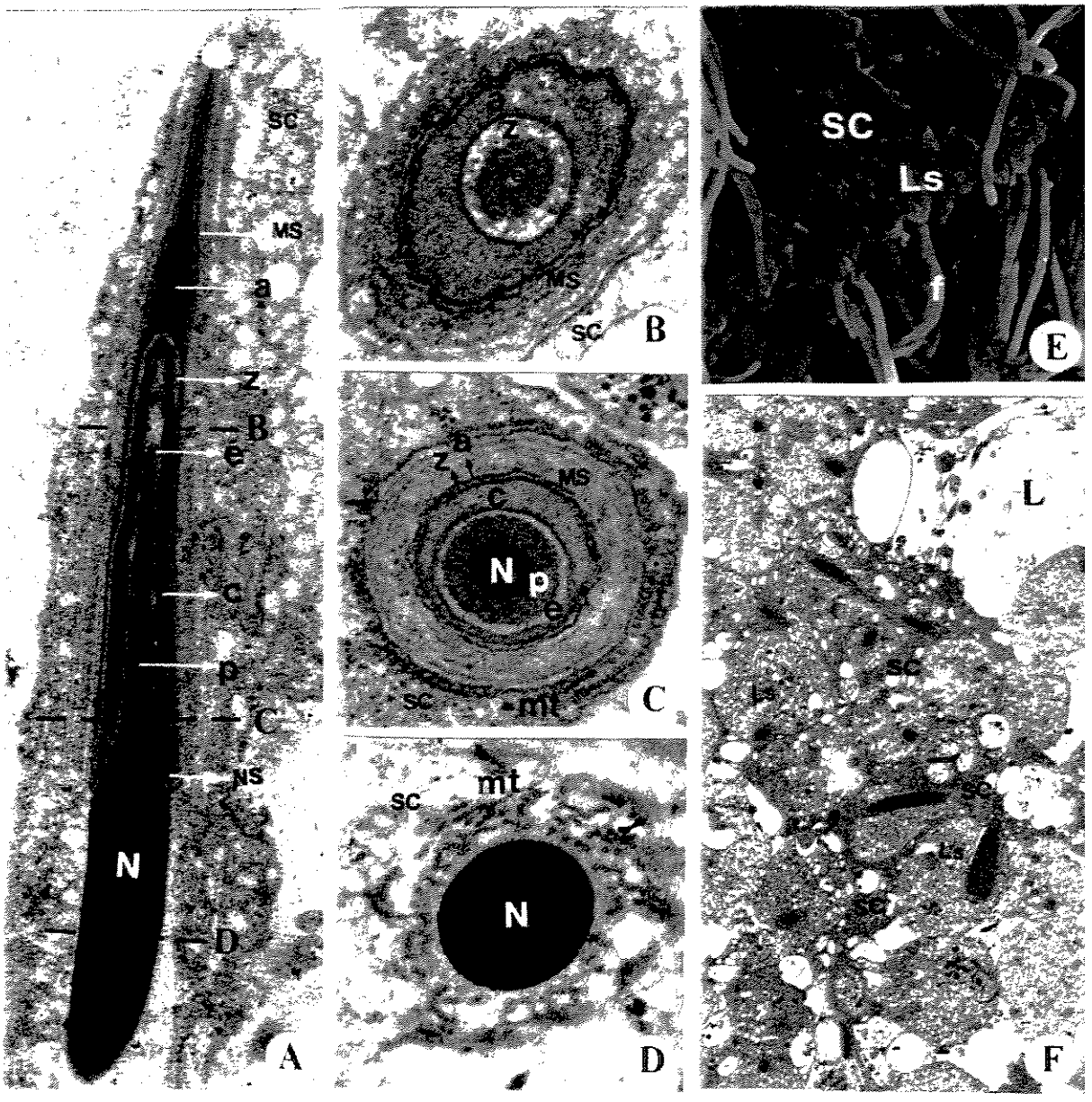
SC
MS
a
z
B
e
C
p
C
NS
N
D
A
z
MS
SC
B
z
B
MS
C
N
p
e
SC
mt
C
SC
mt
N
D
SC
Ls
E
L
SC
Ls
SC
Ls
SC
F

Figure 3

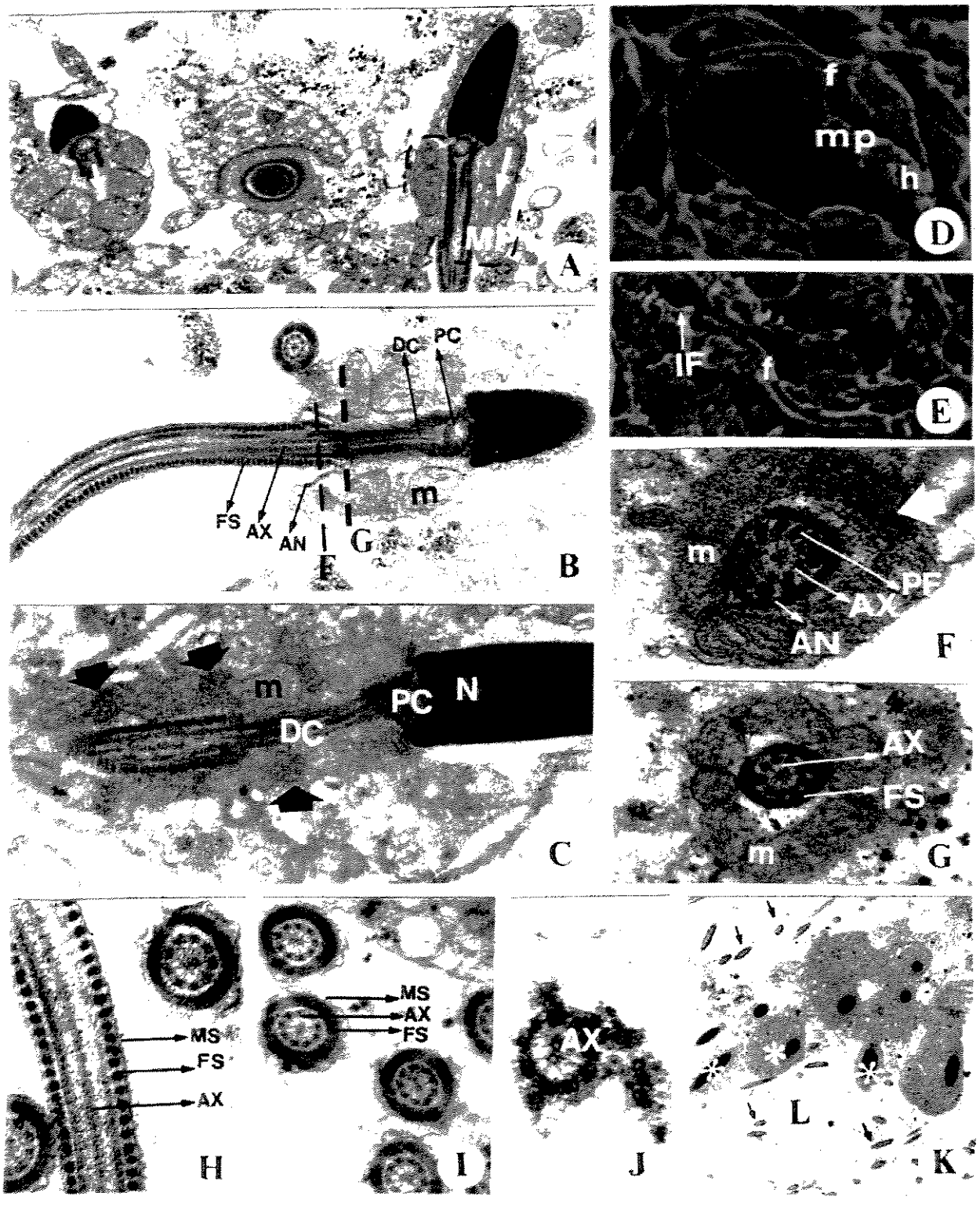
A
f
mp
h
D
DC
PC
IF
f
E
FS
AX
AN
E
G
m
B
m
PC
N
DC
C
m
PF
AX
AN
F
AX
FS
m
G
MS
FS
AX
H
MS
AX
FS
I
AX
J
L
K

Figure 4

LITERATURE CITED

ADAMS, C. S. AND W. E. COOPER. 1988. Oviductal morphology and sperm storage in the keeled earless lizard, *Holbrookia propingua*. Herpetologica 44(2): 190-197.

AL-DOKHI, O. A. 1997. Fine structure of sperm tail differentiation in the sand skink, *Scincus mitranus* (Anderson, 1871) (Squamata: Reptilia). J. Arid Envirom. 37: 395-401.

AL-HAJJ, H., S. JANAKAT AND F. MAHMOUD. 1987. Electron microscopic study of sperm head differentiation in the lizard *Agama stellio*. Can. J. Zool. 65: 2959-2968.

AMARAL, M. J. L. V, A. FERNANDES AND S. M. RECCO-PIMENTEL. 1999. An ultrastructural study of spermiogenesis in three species of Physalaemus (Anura, Leptodactylidae). Biocell 23 (3): 211-221.

ASA, C. S. AND D. M. PHILLIPS. 1987. Ultrastructure of avian spermatozoa: a short review. *In:* Mohri, H. New Horizons in Sperm Cell Research. Tokyo: Japan Scientific Societies Press, p. 365-373.

_____ AND J. STOVER. 1986. Ultrastructure of spermatozoa of the crested tinamou. J. Ultrastruct. Mol. Struct. Res. 94: 170-175.

BACCETTI, B. 1979. The evolution of the acrosomal complex. *In:* Fawcett, D. W. and Bedford, M. The spermatozoon. Baltimore: Urban and Schwarzenberg, p. 305-329.

_____ AND B. A. AFZELLIUS. 1976. The biology of the sperm cell. Monogr. Dev. Biol. 10: 1-124.

_____, E. BIGLIARDI AND A. G. BURRINI. 1980. The morphogenesis of vertebrates perforatorium. J. Ultrastruct. Res. 71: 272-287.

BERGSTROM, B. H. AND J. M. ARNOLD. 1974. Nonkinetochore association of chromatin and microtubules. J. Cell Biol. 62: 917-920.

BOURESLI, M. N., L. F. BISHAY AND N. S. ALZAID. 1981. Observations on the fine structure of the sperm storage crypts in the lizard *Acanthodactylus scutellatus* Hardyi. Arch. Biol. 92 (3): 287-298.

BUTLER, R. D. AND M. S. GABRI. 1984. Structure and development of the sperm head in the lizard *Podarcis* (=*Lacerta*) *taurica*. J. Ultrastruct. Res. 88: 261-274.

CARCUPINO, M., G. CORSO AND M. PALA. 1989. Spermiogenesis in *Chalcides ocellatus tiligugu* (Gmelin) (Squamata: Scincidae): an eletron microscope study. Boll. Zool. 56: 119-124.

CLARK, A. W. 1967. Some aspects of spermiogenesis in a lizard. Amer. J. Anat. 121: 369-400.

CONNER, J. AND D. CREWS. 1980. Sperm transfer and storage in the lizard *Anolis carolinensis*. J. Morphol. 163: 331-348.

COURTENS, J. C. AND A. DEPEIGES. 1985. Spermiogenesis of *Lacerta vivipara*. J. Ultrastruct. Res. 90: 203-220.

CRUZ HÖFLING, M. A. AND C. CRUZ LANDIM. 1978. The fine structure of nuclei during spermiogenesis in the lizard *Tropidurus torquatus* (Lacertilia). Cytologia 43: 61-68.

CRUZ LANDIM, C. AND M. A. CRUZ HÖFLING. 1977. Electron microscope study of lizard spermiogenesis in *Tropidurus torquatus* (Lacertilia). Caryologia 30: 151-162.

CUELLAR, O. 1966. Oviductal anatomy and sperm storage structures in lizards. J. Morphol. 119: 7-20.

DEL CONTE, E. 1976. The subacrosomal granule and its evolution during spermiogenesis in a lizard. Cell Tiss. Res. 171: 483-498.

DUFAURE, J. P. 1971. L'ultrastructure du testicule de Lézard vivipare (Reptile: Lacertilien). II. Les cellules de Sertoli. Etude du glycogene. Z. Zellforsch 115: 565-578.

FAWCETT, D. W. 1970. A comparative view of sperm ultrastructure. Biol. Reprod. (Suppl.) 2: 90-127.

_____ AND D. M. PHILLIPS. 1970. Recent observations on the ultrastructure and development of the mammalian spermatozoon. *In:* BACCETTI, B. Comparative spermatology. Rome: Accademia Nazionale dei Lincei, p. 13-28.

_____, W. A. ANDERSON AND D. M. PHILLIPS. 1971. Morphogenetic factors influencing the shape of the sperm head. Develop. Biol. 26: 220-251.

FOX, W. 1963. Special tubules for sperm storage in female lizards. Nature 198: 500-501.

FURIERI, P. 1970. Sperm morphology in some reptiles: Squamata and Chelonia. *In:* BACCETTI, B. (edit). Comparative spermatology (Procceding of the International Symposium) Rome: Accademia Nazionale dei Lincei, pp. 115-143.

_____ 1974. Spermi e spermatogenesi in alcuni iguanidi argentini. Riv. Biol. 67: 233-279.

_____ 1975. The peculiar morphology of the spermatozoon of *Bombina variegata* (L.). Monitore Zool. Ital. (New series) 9: 185-201.

GURAYA, S. S. 1971. Histochemical observations on the lizard spermiogenesis. Acta Anat. 79: 270-279.

HALPERT, A. P., W. R. GARSTKA AND D. CREWS. 1982. Sperm transport and storage and its relation to the annual sexual cycle of the female red-sided garter snake, *Thamnophis sirtalis parietalis*. J. Morphol. 174 (2): 149-159.

HAMILTON, D. W. AND D. W. FAWCETT. 1968. Unusual features of the neck and middle-piece of snake spermatozoa. J. Ultrastruct. Res. 23: 81-97.

HARDING, H. R., K. P. APLIN AND M. MAZUR. 1995. Ultrastructure of spermatozoa of australian blindsnakes, *Ramphotyphlops* spp. (Typhlopidae, Squamata): first observations on the mature spermatozoon of scolecophidian snakes. *In:* JAMIESON, B. G. M., J. AUSIO AND J. L. JUSTINE. (eds), Advances in Seprmatozoal Phylogeny and Taxonomy. Mém. Mus. Natn. Hist. Nat. 166: 385-396.

HEALY, J. M. AND B. G. M. JAMIESON. 1992. Ultrastructure of spermatozoon of the tuatara (*Sphenodon punctatus*) and is relevance to the relationships of the Sphenodontida. Phil. Trans. R. Soc. Lond. B 335: 193-205.

_____ 1994. The ultrastructure of spermatogenesis and epididymal spermatozoa of the tuatara *Sphenodon punctatus* (Sphenodontida: Amniota). Phil. Trans. R. Soc. Lond. B 344: 187-199.

HUMPHREYS, P. N. 1975. The differentiation of the acrosome in the spermatid of the budgerigar (*Melopsittacus undulatus*). Cell Tissue Res. 156: 411-416.

JAMIESON, B. G. M. 1995. The ultrastructure of spermatozoa of the Squamata (Reptilia) with philogenetic coniderations. *In:* _____, J. AUSIO AND J. L. JUSTINE. Advances in spermatozoal phylogeny and Taxonomy. Paris: Édition du muséum national d'histoire naturelle, p. 395-383.

_____ AND J. M. HEALY. 1992. The phylogenetic position of the tuatara, *Sphenodon* (Sphenodontida: Aminiota), as indicated by cladistic analysis of the ultrastructure of spermatozoa. Phil. Trans. R. Soc. Lond. B 335: 207-219.

_____ AND D. M. SCHELTINGA. 1993. The ultrastructure of spermatozoa of *Nangura spinosa* (Scincidae, Reptilia). Mem. Queensland Mus. 34: 169-179.

_____, S. C. OLIVER AND D. M. SCHELTINGA. 1996. The ultrastructure of the spermatozoa of Squamata. I. Scincidae, Gekkonidae and Pygopodidae (Reptilia). Acta Zool. (Stockholm) 77(1): 85-100.

_____, D. M. SCHELTINGA AND A. D. TUCKER. 1997. The ultrastructure of spermatozoa of the australian freshwater crocodile, *Crocodylus johnstoni* Krefft, 1873 (Crocodylidae, Reptilia). J. Submicr. Cytol. Pathol. 29: 265-274.

KRAUSE, W. J. 1996. Meiosis and male reproductive organs. *In:* _____. Essentials of humam histology. New York: Little, Brown and Company, p. 331-352.

MYLES, D. G. AND P. K. HEPLER. 1977. Spermiogenesis in the fern *Marsilea*: microtubules, nuclear shaping and cytomorphogenesis. J. Cell Sci. 23: 57-83.

NAGANO, T. 1962. Observations on the fine structure of the developing spermatids in the domestic chicken. J. Cell Biol. 14: 193-205.

NEWTON, W. D. AND S. E. TRAUTH. 1992a. Sperm morphology and ultrastructure in *Cnemidophorus sexlineatus* (Sauria: Teiidae). Am. Soc. Zool. (30): 44A.

_____ 1992b. Ultrastructure of the spermatozoon of the lizard *Cnemidophorus sexlineatus* (Sauria: Teidae). Herpetologica 48: 330-343.

OLIVER, S. C., B. G. M. JAMIESON AND D. M. SCHELTINGA. 1996. The ultrastructure of spermatozoa of Squamata. II. Agamidae, Varanidae, Colubridae, Elapidae and Boidae (Reptilia). Herpetologica 52: 216-241.

PHILLIPS, D. M. 1970. Ultrastructure of spermatozoa of the wooly opossum *Caluromys philander*. J. Ultrastruc. Res. 33: 381-397.

_____ 1972. Comparative analysis of mammalian sperm motility. J. Cell. Biol. 53: 561-573.

_____ AND C. S. ASA. 1993. Strategies for formation of the midpiece. *In:* BACCETTI, B. Comparative spermatology 20 years after, p. 997-1000. New York: Raven Press.

_____ AND G. OLSON. 1975. Mammalian sperm motility and structure in relation to function. *In:* AFZELLIUS, B. A. The functional anatomy of the spermatozoon, p. 117-126. Oxford: Pergamon Press.

SAITA, A.; COMAZZI, M. AND PERROTA, E. 1987. Electron microscope study of spermiogenesis in *Caimam crocodylus* L. Boll. Zool. 4: 307-318.

_____ 1988. New data at the E. M. on the spermiogenesis of *Iguana delicatissima* (Laurent) involving comparative significance. Acta Embryol. Morphol. Exper. n. s. 9: 105-114.

SHIROYA, Y., H. HOSOYA, I. MABUCHI AND Y. T. SAKAI. 1986. Actin filament in the acrosome of abalone spermatozoa. J. Exp. Zool. 239: 105-115.

SOLEY, J. T. 1994. Centriole development and formation of the flagellum during spermiogenesis in the ostrich (*Struthio camelus*). J. Anat. 185: 301-313.

SPRANDO, R. L. AND L. D. RUSSEL. 1988. Spermiogenesis in red-ear turtle (*Pseudemys scripta*) and the domestic fowl (*Gallus domesticus*): a study of cytoplasmic elimination. J. Morphol. 198: 95-118.

SRINIVAS, S. R., T. SHIVANANDAPPA, S. N. HEGDE AND H. B. D. SARKAR. 1995. Sperm storage in the oviduct of the tropical rock lizard, *Psammophilus dorsalis*. J. Morphol. 224 (3): 293-301.

TEIXEIRA, R. D., G. H. C. VIEIRA, G. R. COLLI AND S. N. BAO. 1999a. Ultrastructural study of spermatozoa of the neotropical lizards, *Tropidurus semiaeniatus* and *Tropidurus torquatus* (Squamata: Tropiduridae). Tissue & Cell 31: 308-317.

_____, G. R. COLLI AND S. N. BÁO. 1999b. The ultrastructure of the worm lizard *Amphisbaena alba* (Squamata, Amphisbaenidae) and the phylogenetic relationships of amphisbaenians. Canad. J. Zool. – Rev. Canad. Zool. 77: 1254-1264.

YASUZUMI, G. AND M. YASUDA. 1968. Spermatogenesis in animals as revealed by eletron microscopy. XVIII. Fine structure of developing spermatids of the Japanese

freshwater turtle fixed with potassium permanganate. Z. Zellforsch. Mikrosk. Anat. 85: 18-33.

7. CONCLUSÕES FINAIS

- ✓ O ciclo reprodutivo da espécie *I. iguana* é anual, e o período de máximo desenvolvimento dos testículos é relativamente curto, ocorrendo nos meses de Julho a Setembro. É importante reforçar que a literatura pertinente demonstra que lagartos (mesmo em regiões tropicais) têm o período reprodutivo no verão. As altas temperaturas são consideradas por muitos o fator estimulador da espermatogênse. Para esta espécie, o ciclo sazonal de cheias e secas estimulou uma adaptação visando um período adequado para a formação de casais para a cópula, e um posterior período adequado à gestação e desenvolvimento embrionário.

- ✓ O processo de espermiogênese da espécie *I. iguana* envolve uma série de modificações ultra-estruturais, que culminam na produção de organelas necessárias ao espermatozóide. Acredita-se que os espermatozóides liberados no lumen dos túbulos seminíferos apresentam todas as suas características morfológicas bem definidas. No entanto para esta espécie os espermatozóides liberados na espermiação não estão maduros. Essa conclusão pode ser tomada com base nos corpos densos em formação na peça intermediária também pela presença de citoplasma em torno da cabeça de espermatozóides livres no lumen dos túbulos seminíferos.