# FORMATION OF THE SPERMATOZOON DURING AND AFTER SPERMIOGENESIS IN *Iguana iguana* LINNAEUS, 1758 (Reptilia, Iguanidae), WITH SPECIAL ATTENTION TO THE MIDDLE PIECE AND AXONEMIC COMPLEX

## *FORMAÇÃO DO ESPERMATOZÓIDE DURANTE E APÓS A ESPERMIOGÊNESE EM* Iguana iguana *LINNAEUS, 1758 (Reptilia, Iguanidae), COM ESPECIAL ATENÇÃO PARA A PEÇA INTERMEDIÁRIA E COMPLEXO AXONÊMICO*

**Adelina FERREIRA[1]**

1. Professora, Doutora, Instituto de Biociências, Departamento de Biologia e Zoologia, Universidade Federal de Mato Grosso, Cuiabá, MT, Brasil. adelina@ufmt.br

**ABSTRACT:** Spermiogenesis involves a series of cellular modifications, culminating in the production of a highly specialized cell, the spermatozoon. To reassess the peculiarities of this process, specimens of the lizard *Iguana iguana* were collected in the Pantanal, Mato Grosso do Sul state, Brazil; the testis and ducts were processed according to routine methods for light, scanning and transmission electron microscopy. After nuclear condensation and elongation, the cytoplasm and organelles flow towards the basal area, below the nucleus. An infolding occurs at the nuclear base, where the middle piece is implanted. This area contains a centriole pair, with the proximal one perpendicular and the distal one parallel to the nuclear axis. The distal centriole can be functionally compared to the basal corpuscle at the axoneme base. The mitochondria, that are round or elongated with transverse cristae, initially organized around the centriole in continuous rings; later they alternate with dense bodies. Thus the dense bodies are apparently formed by mitochondrial modification with deposition of dense material. In *I. iguana* the formation of dense bodies is one of the last events of spermiogenesis. Marking the transition between middle piece and flagellum is the annulus. This structure blocks the displacement of mitochondria from the middle piece during flagellar movement. Halfway down the middle piece, a fibrous sheath is formed by the accumulation of an amorphous layer surrounding the axoneme, and this structure is the axonemic complex. In the spermatozoa of *I. iguana*, the flagellar end piece is reduced in diameter, because the fibrous sheath terminates and only the typical axonemal microtubule pattern (9+2) is present. A large amount of cytoplasm is observed around spermatozoa in the seminiferous tubule lumen, suggesting that these are "immature" spermatozoa. Structural modifications occur during their passage through the reproductive tract. These post-testis modifications have not been precisely located along the reproductive tract and are probably not essential for spermatozoon mobility. Some features of the male reproductive tract are shown.

**KEYWORDS:** Reproduction. Lizard. Ultrastructure. Seminal receptacle.

## INTRODUCTION

The modification of testicular germ cells to give rise to spermatozoa in lizards has already been explored (JAMIESON, HEALY, 1992; HEALY, JAMIESON, 1994; JAMIESON, 1995; JAMIESON et al., 1996; OLIVER et al., 1996). The reproductive tract contributes with the production of secretion and the final maturation of spermatozoa in lizards (DUFAURE, SAINT GIRONS, 1984; DEPEIGES et al., 1985; DUFAURE et al., 1986; HAIDER, RAI, 1986; RAVET et al., 1987; COURTY, 1991). However, this process has not been detailed for *Iguana iguana*, with an important description of the spermatozoon for Vieira et al. (2004). This important representative of the Iguanidae family has ample distribution in neotropical regions (ZUG, 1997) and is representative of the Brazilian Pantanal fauna. The lizard is large, with the young measuring about 15 centimeters while adults reach 2 meters in length (FERREIRA et al., 2002a). They present a peculiar seasonal reproductive cycle adapted to the environmental conditions of the Pantanal region (FERREIRA et al., 2002a).

The spermiogenesis of this species includes a few more complicated structures, described in Lacertilia, usually known as the Acrosomal complex and the Axonemic complex (FERREIRA, 2002b). The acrosomal complex, is made up of the acrosome, the perforatorium and other associated structures, which coat the apical surface of the spermatozoon head (FERREIRA et al., 2002b). Here, the reproductive process was investigated to describe the axonemic complex, including features of the final stage of spermiogenesis, as well as the reproductive duct’s epithelium.

## MATERIAL AND METHODS

Specimens of the lizard *Iguana iguana* were collected in the Pantanal of Mato Grosso do Sul (14°22°S and 53°61°W) Brazil. They were anesthetized with ethyl ether inhalation, the testis and reproductive tract removed, fixed and processed according to routine methods for scanning (SEM) and transmission electron microscopy (TEM) and for light microscopy (LM), stained with hematoxylin-eosin and periodic acid Schiff (PAS).

## RESULTS AND DISCUSSION

During the reproductive period of *Iguana iguana*, seminiferous tubules contain lots of germ cells in different developmental stages and a large amount of spermatozoa in the lumen (Figs 1A, 1B). During spermiogenesis one of the more complicated structures is the axonemic complex, formed by the middle piece and flagellum (Fig. 1C). A pair of centrioles is found in the contact region of head and flagellum (Fig. 1D), the proximal one lying perpendicular while the distal one is parallel to the longer spermatozoon axis (Figs. 1E, 1H). These centrioles are similar to the basal corpuscles of cilia in that they organize the microtubule arrangement of the axoneme (JAMIESON, HEALY, 1992; JAMIESON, 1995; ALBERTS et al., 1994). Completing the middle piece a number of mitochondria are directed towards this region surrounding the centrioles (Fig. 1F) in a circular arrangement (Fig. 1E, 1G, 1H, 1J). Between this ring of mitochondria and the initial portion of the axoneme, a cylindrical layer of peripheral fibers appears (Fig 1E, 1J). The last structures to be formed in the middle piece are the dense bodies between the mitochondria (Figs. 1G, 1H, 1J). The middle piece is limited by a ring constricting the cytoplasm known as annulus (Figs. 1E, 1J). The middle piece in lizards varies greatly in size (JAMIESON, 1995; OLIVER et al., 1996). Also, the order of formative events is variable and some authors describe dense bodies formed previous to the mitochondria alignment around centrioles (PHILLIPS, ASA, 1993). The flagellar axoneme has the typical 9+2 microtubule arrangement (Fig. 1J, 1K). Extending along the axoneme is a fibrous sheath made up of regularly distributed separate rings (Figs. 1E, 1K). The fibrous sheath rings are electron dense and amorphous, covered externally by the plasma membrane. Elastic properties have been attributed to the sheath, which would give it an important function in spermatozoon mobility (FAWCETT, 1970; SOLEY, 1994; JAMIESON, 1995, FERREIRA, DOLDER, 2003). In the final flagellar region the fibrous sheath progressively reduces its thickness so that the final portion of the flagellum consists only of the axoneme (Fig. 1L). This feature is common to various lizards (JAMIESON et al., 1996, TEIXEIRA et al., 1999, FERREIRA, DOLDER, 2003). The spermatozoa eliminated into the seminiferous tubule lumen still present a large amount of cytoplasm around the head region but not in the flagellum (Fig. 1I). This feature has not been previously reported in the literature (FERREIRA et al., 2002a).

The reproductive tract epithelium contains secretory and basal cells. During the reproductive period the epithelium thickness varies noticeably depending on the size of these cells and the amount of secretion produced (Figs. 2A, 2B). Cell secretions consist of PAS-positive glycoproteins and glycosaminoglycans (Figs. 2D, 2E) contained in large vesicles (Figs.s 2C, 2E, 2F). Secretory regions carry out a function similar to mammal seminal vesicle and prostate (DUFAURE et al., 1986, FERREIRA, DOLDER, 2007). This is common in other lizards, with variations in the intensity and granule volume (DEPEIGES, DUFAURE, 1977; DUFAURE, SAINT GIRONS, 1984; DEPEIGES et al., 1985; HAIDER, 1985). This secretion is responsible for an increase in volume of spermatic fluid, permitting mobility and preventing spermatozoon dispersal (DUFAURE, SAINT GIRONS, 1984; DUFAURE et al., 1986; MESURE et al., 1991, FERREIRA, DOLDER, 2003; FERREIRA, DOLDER, 2007). It is only in the reproductive period that the duct’s epithelium reaches its maximum height, as well as maximum activity (KOHANE et al., 1980; MESURE et al., 1991).

In the epididymis dense bodies in the middle piece reach their final shape (Figs. 2G, 2H, 2I). The final duct region widens to form a glandular structure called the seminal receptacle (Figs. 2J, 2L), which also produces an intense glycoprotein secretion (Fig. 2K). This structure is common in lizards (CONNER, CREWS, 1980; KUMARI et al., 1990; SRINIVAS et al., 1995; TOKARZ, 1999; FERREIRA, DOLDER, 2007) and snakes (FOX, 1956; SCHAEFER, ROEDING, 1973; HALPERT et al., 1982) and is responsible for the storage and final spermatozoon characterization, adding surface glycides and probably antigens, essential to successful reproduction (CONNER, CREWS, 1980; ADAMS, COOPER, 1988; OLSSON et al., 1994; FERREIRA, DOLDER, 2003).

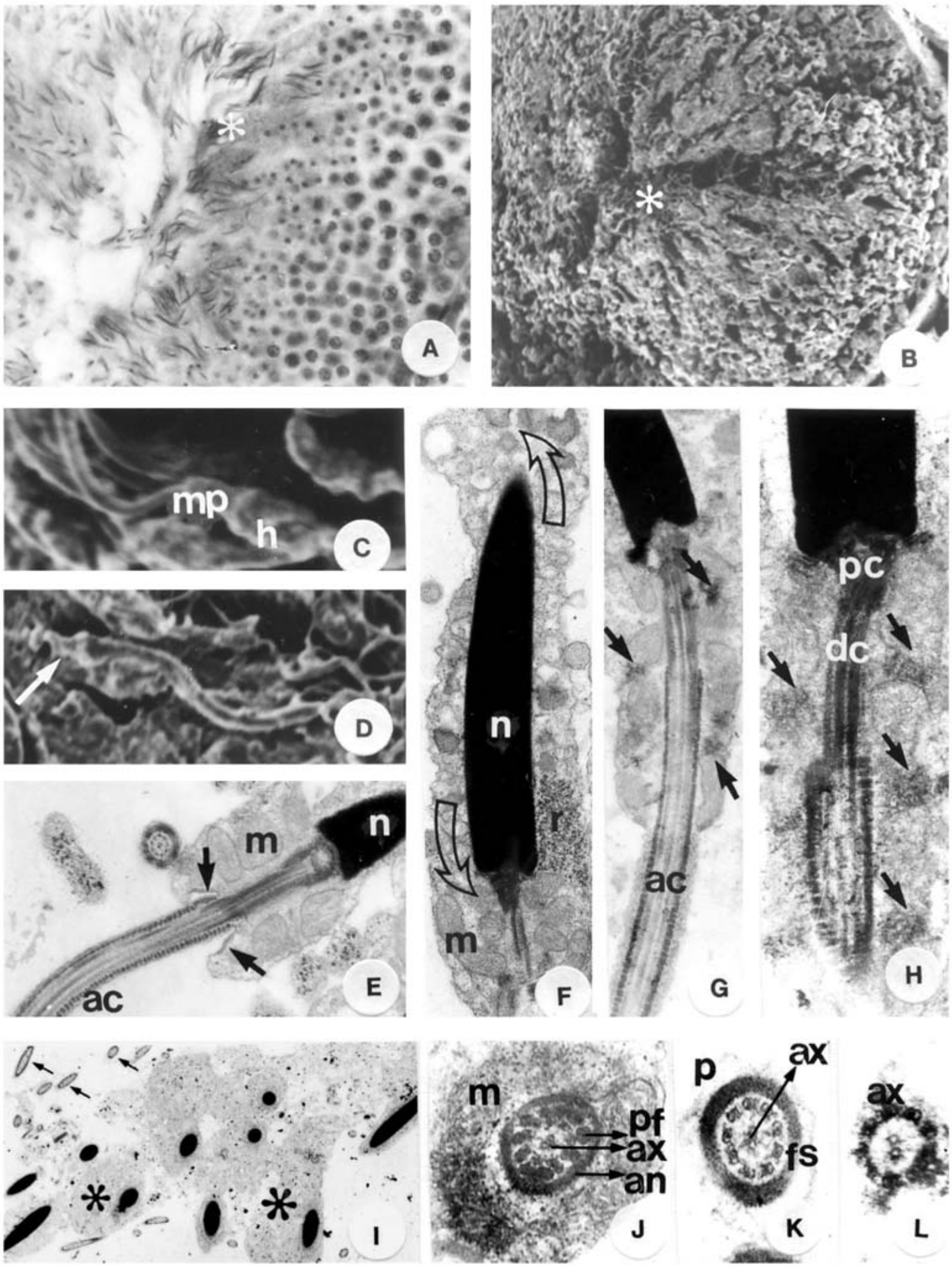


**Figure 1**: **A.** and **B.** Seminiferous tubules viewed during the reproductive cycle of *Iguana iguana*, with the presence of developing germ cells and many spermatozoa (asterisks). **A.** Light microscopy (LM), hematoxylin-eosin stained. X600. **B.** Scanning Electron Microscopy (SEM) X800. **C.** and **D.** SEM of spermatozoa. Head region (h), middle piece (mp). Arrow indicates the flagellum implantation region. X8000. **E-L:** Transmission Electron Microscopy (TEM) of advanced spermatids and spermatozoa in the lumen of seminiferous tubules. **E.** Nucleus (n), mitochondria (m) forming rings around proximal and distal centrioles; middle piece limited by the annulus (arrow), and flagellum formed by the axonemic complex (ac). X14000. **F.** The arrows indicate the streaming of residual cytoplasm towards the tip and mitochondria (m) to the middle piece; ribosomes (r). X 12000. **G.** Initial formation of dense bodies between mitochondria (arrows). Dense fibers of the axonemic complex (ac) appear half may down the middle piece . X16000. **H.** Slightly dense bodies (arrows) around the proximal centriole (pc) and distal centriole (dc). X18000. **I.** Spermatozoa in the seminiferous tubule lumen, with a great amount of cytoplasm around the head (asterisks) but none around the flagellum (arrows). X3000 **J., K.** and **L.** Transverse sections of different regions of the axonemic complex: **J.** Middle piece: in the center the axoneme (ax), is surrounded by the peripheral fibers (pf), annulus (an), mitochondria (m) and dense body (arrow). X48000. **K.** Principal part of the flagellum including axoneme (ax), surrounded by the fibrous sheath and plasma membrane (p). X64000. **L.** Terminal part of flagellum consisting in only the axoneme (ax). X72000.

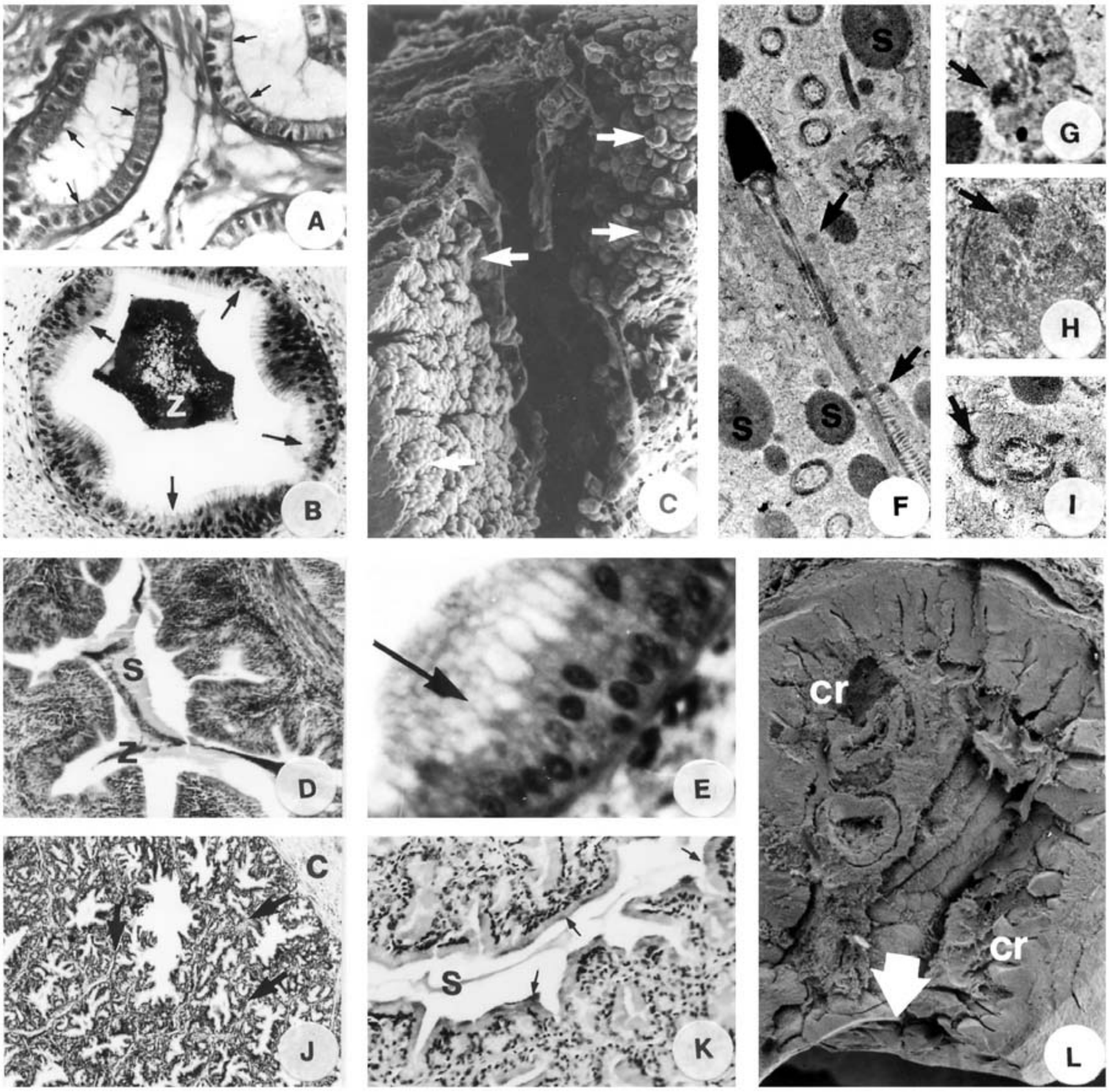


**Figure 2:** **A.** and **B.** LM of the epididymis of *Iguana iguana,* hematoxylin-eosin stained. X400. **A.** At the beginning of the reproductive cycle, with reduced secretion (arrows) and absence of spermatozoa in the lumen. **B.** In the reproductive period, with many spermatozoa (z) in the lumen well and developed secretory cells (arrows). **C.** SEM of the epididymis in the reproductive period. The arrows indicate the abundance of large secretory vesicles. X500. **D.** Final region of the epididymis, with spermatozoa (z) surrounded by PAS positive secretion (s). X200. **E.** Detail of the epididymal epithelium, the arrow indicates secretion vesicles. X1000. **F.** TEM spermatozoon in the epididymis; arrows indicate the dense bodies, a little more developed. Around the spermatozoon are many secretory vesicles (s). X12000. **G. H.** and **I.** Transverse sections of the middle piece where arrows indicate the dense bodies in stages of differentiation. X40000 **J.** LM of seminal receptacle, showing the glandular aspect, supported by connective tissue septae (arrows), branching out from the capsule (c). X100. **K.** Demonstration of PAS positive secretion (s) and apical region epithelial cells of the seminal receptacle (arrows). X400. **L.** SEM of the seminal receptacle. The arrow indicates the aperture region of the cloaca. Internally, the organ forms crypts (cr) where the spermatozoa are stored until copulation. X200.

## CONCLUSION

In conclusion, during spermiogenesis in *I. iguana* the basic structures necessary for spermatozoon function are formed. The loss of excessive cytoplasm as well as the completion of the middle piece dense bodies apparently occurs during the passage though the reproductive ducts. The duct cells and those of the seminal receptacle produce a great amount of secretion that contributes to the final spermatozoon maturation. Glycoproteins secreted by the duct's epithelial cells are probably added to the spermatozoon surface.

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**RESUMO:** A espermiogênese envolve uma série de modificações celulares, culminando na formação de uma célula altamente especializada, o espermatozóide. Para re-avaliarmos as peculiaridades desse processo, espécimes do lagarto *Iguana iguana* foram coletados no Pantanal, estado de Mato Grosso do Sul, Brasil; os testículos e ductos foram retirados e processados de acordo com a rotina para microscopia de luz e eletrônica de transmissão e varredura. Após a condensação e alongamento do núcleo, o citoplasma e as organelas se deslocam para região basal abaixo do núcleo. Nessa região basal do núcleo é implantada a peça intermediária. Essa área contém um par de centríolos, sendo um proximal e perpendicular e um distal paralelo ao eixo maior do núcleo. O centríolo distal pode ser comparado funcionalmente ao corpúsculo basal na base do axonema. As mitocôndrias são redondas ou alongadas com cristas transversais, organizadas em torno dos centríolos em anéis contínuos; esses anéis são alternados por corpos densos. Esses corpos densos são aparentemente formados por mitocôndrias modificadas com deposição de material denso. Em *I. iguana* a formação desses corpos densos é um dos últimos eventos da espermiogênese. O que marca a transição entre a peça intermediária e o flagelo é o annulus. Esta estrutura obstrui o deslocamento das mitocôndrias da peça intermediária durante o movimento flagelar. A peça intermediária apresenta uma parte incompleta formada pelo acúmulo de uma camada amorfa que cerca o axonema chamada de bainha fibrosa, o conjunto dessas estruturas é o complexo axonêmico. No espermatozóide de *I. iguana* a peça final flagelar tem o diâmetro reduzido, com o término da bainha fibrosa permanecendo um típico padrão axonêmico de microtúbulos (9+2). Uma quantidade grande de citoplasma é observada em torno dos espermatozóides no lúmen dos túbulos seminíferos, sugerindo que estes são "espermatozóides imaturos". As modificações estruturais ocorrem durante a sua passagem através do trato reprodutivo. Estas modificações do espermatozóide após ter saído do testículo não foram determinadas precisamente ao longo do trato reprodutivo e não são provavelmente essenciais para a mobilidade do espermatozóide. Algumas características do trato reprodutivo dos indivíduos machos são mostradas.

**PALAVRAS - CHAVE:** Reprodução. Lagarto. Ultra-estrutura. Receptáculo seminal.

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