Ultrastructure of Spermiogenesis in the American Alligator, *Alligator mississippiensis* (Reptilia, Crocodylia, Alligatoridae)

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**ABSTRACT** Testicular samples were collected to describe the ultrastructure of spermiogenesis in *Alligator mississippiensis* (American Alligator). Spermiogenesis commences with an acrosome vesicle forming from Golgi transport vesicles. An acrosome granule forms during vesicle contact with the nucleus, and remains posterior until mid to late elongation when it diffuses uniformly throughout the acrosomal lumen. The nucleus has uniform diffuse chromatin with small indices of heterochromatin, and the condensation of DNA is granular. The subacrosome space develops early, enlarges during elongation, and accumulates a thick layer of dark staining granules. Once the acrosome has completed its development, the nucleus of the early elongating spermatid becomes associated with the cell membrane flattening the acrosome vesicle on the apical surface of the nucleus, which aids in the migration of the acrosomal shoulders laterally. One endonuclear canal is present where the perforatorium resides. A prominent longitudinal manchette is associated with the nuclei of late elongating spermatids, and less numerous circular microtubules are observed close to the acrosome complex. The microtubule doublets of the midpiece axoneme are surrounded by a layer of dense staining granular material. The mitochondria of the midpiece abut the proximal centriole resulting in a very short neck region, and possess tubular cristae internally and concentric layers of cristae superficially. A fibrous sheath surrounds only the axoneme of the principal piece. Characters not previously described during spermiogenesis in any other amniote are observed and include (1) an endoplasmic reticulum cap during early acrosome development, (2) a concentric ring of endoplasmic reticulum around the nucleus of early to middle elongating spermatids, (3) a band of endoplasmic reticulum around the acrosome complex of late developing elongate spermatids, and (4) midpiece mitochondria that have both tubular and concentric layers of cristae. J. Morphol. 271:1260–1271, 2010.

**KEY WORDS:** spermatids; alligator; ultrastructure; spermiogenesis; acrosome

**INTRODUCTION**

Members of the Crocodylia are large, oviparous, and aquatic (fresh, brackish, or salt water) vertebrates distributed in subtropic and tropic zones (Ferguson, 1985). Males copulate with females via insertion of a single penis into the female cloaca (Gadow, 1887), and sperm is stored in the female oviduct (Gist et al., 2008; Bagwill et al., 2009) until ovulation. Eggs are deposited in a hole or mound (Thorbjarnarson, 1996) and sex is determined by nest temperature in all species of Crocodylia investigated (Lang and Andrews, 1994).

Based on morphological data, extant Crocodylia can be broken up into the Alligatoroidea (alligators and caiman), Crocodylinae (crocodiles), Tomistominae (false gavial), and Gavialinae (true gavials; Gatesy et al., 2004).

Mature sperm ultrastructure has been described in two Crocodylia members, *Caiman crocodylus* (spectacled caiman; Saita et al., 1987) and *Crocodylus johnsoni* (freshwater crocodile; Jamieson et al., 1997). Ultrastructural changes that occur during spermiogenesis within Crocodylia testes have been described for *C. crocodylus* (Saita et al., 1987) and *Alligator sinensis* (Chinese alligator; Wang et al., 2008). No additional ultrastructural investigations exist on the sperm structure or spermiogenic stages of other Crocodylia taxa; however, Gribbins et al. (2006) evaluated the cytology of spermatogenesis in *Alligator mississippiensis* (American alligator) in an effort to describe the germ cell developmental strategy of this species. Also, Moore et al. (2009) illustrates the development of the testes in post-hatching juveniles.
Interestingly, unlike their fellow Archosaurs (birds), A. mississippiensis and A. sinensis have a germ cell development strategy more similar to other members of the paraphyletic Reptilia (Gribbins and Gist, 2003; Gribbins et al., 2003, 2005, 2008; Wang et al., 2008; Rheubert et al., 2009), and thus, A. mississippiensis has a pleiomorphic-like (temporal) germ cell development strategy.

While sperm morphology is an invaluable tool for comparative and phylogenetic analyses (review: Jamieson, 1995), few studies have investigated the effects of aquatic environmental toxicants on sperm development in amniotic models. It is well established that baseline data on the ultrastructural changes during spermiogenesis are necessary to understand how potential reproductive toxins (e.g., pesticides; Russell et al., 1990) alter spermatogenesis in vertebrates. These types of studies may provide data on sperm abnormalities upon exposure to man-made toxins and may be integral in future species conservation. Considering the affinity of members of the Crocodylia to water, these reptiles may represent sentinel species for histopathological studies on how potential aquatic reproductive toxicants, such as pesticides, affect spermatogenic output in the testis of semi-aquatic reptiles.

Here we describe the ultrastructure of the spermiogenic stages in Alligator mississippiensis. We then compare these data with those published for Caiman crocodylus (Saita et al., 1987) and Alligator sinensis (Wang et al., 2008). We will also note any similarities or differences between alligator spermatids and the products of spermiogenesis within the testes of the Tuatara (Sphenodon punctatus; Healy and Jamieson, 1994), lizards (Clark, 1967; Dehlawi et al., 1992; Gribbins et al., 2003; Rheubert et al., in press), chelonians (Sprando and Russell, 1988; Healy and Jamieson, 1992; Al-Dokhi and Al-Wasel, 2001a,b, 2002; Zhang et al., 2007), and birds (Lin and Jones, 1993; Soley, 1997; Aire, 2007).

MATERIALS AND METHODS

Animal Collection

Five adult male American Alligators (Alligator mississippiensis) between 2.5 and 3 m were collected during May 2002 and May 2003 (peak month of spermiogenesis: Gribbins et al., 2006) from the Rockefeller Wildlife Refuge in Grand Chenier, LA. Alligators were captured by noose or by baited hooks that were placed in canals within the refuge. Upon capture, alligators were sacrificed and the testes were removed immediately by dissection. The testes were submerged in Trump’s Fixative (EMS, Hatfield, PA) and minced thoroughly into small pieces. The testicular pieces were then placed in fresh Trump’s fixative and stored under refrigeration for at least 48 h (4°C).

Tissue Preparation

Testicular tissues cut into 2-3 mm blocks were washed twice with cacodylate buffer (pH 7.0) for 20 min each. They were then post-fixed in 2% osmium tetroxide for 2 h, washed with cacodylate buffer (pH 7.0) three times for 20 min each, dehydrated in a graded series of ethanol (70%, 85%, 95%, X2, 100% X2), and cleared twice with 10 min treatments of propylene oxide. Each piece of alligator testis was then gradually introduced to epoxy resin (Embed 812, EMS, Hatfield, PA) (2:1 and 1:1 solutions of propylene oxide: epoxy resin). Testicular tissues were then placed in pure Embed 812 for 24 h. Fresh resin was prepared and the tissues were embedded in small beam capsules, and subsequently cured for 48 h at 70°C in a Fisher isostatic vacuum oven (Fisher Scientific, Pittsburg, PA). Sections (90 nm) were obtained by use of a diamond knife (DDK, Wilmington, DE) on an LKB automated ultramicrotome (LKB Produkter AB, Bromma, Sweden). Sections were then placed on copper grids and stained with uranyl acetate (18 min) and lead citrate (5 min).

Ultrastructural Analysis

Testicular samples were viewed using a Jeol JEM-1200EX II transmission electron microscope (Jeol). Micrographs were taken of representative spermatids and structural components associated with spermiogenesis via a Gatan 785 Erlangshen digital camera (Gatan, Warrendale, PA). The micrographs were then analyzed and composite plates were assembled using Adobe Photoshop CS (Adobe Systems, San Jose, CA).

RESULTS

Spermatids develop within the apical portion of the seminiferous epithelium (Fig. 1) of the seminiferous tubules of Alligator mississippiensis as described previously by Gribbins et al. (2006). Once spermiogenesis is completed mature spermatozoa are shed into the lumina of the seminiferous tubules for transport to the excurrent duct system. The beginning of spermiogenesis is marked by the accumulation of round spermatids within the seminiferous epithelium of A. mississippiensis upon the completion of meiosis. During these early stages, an acrosome vesicle forms (Fig. 2A,B, Av), and a juxtapositioned Golgi apparatus (Fig. 2C, black arrowhead) dominates the spermatid cytoplasm near the apex of the nucleus. Budding from the most proximal cisternum of the Golgi are transport vesicles (Fig. 2C, white arrowhead) that merge with the developing acrosome and are responsible for its increase in size during the round spermatid stage. The acrosome vesicle is not in contact with the nuclear membrane during the vesicle’s early development (Fig. 2A), and an acrosome granule is not seen within the vesicle until contact has been made with the nucleus (Fig. 2B, white arrowhead).

The cytoplasm at this time is packed with mitochondria (Fig. 2A, white arrows) and abundant endoplasmic reticula (Fig. 2A, SR). As the acrosome grows in size, it collapses and flattens the apical surface of the round spermatid nucleus at the climax of round spermatid development (Fig. 2D, Av) and at this point the subacrosome space can be observed (Fig. 2D, black arrowhead). The most unusual feature of the early acrosome phase of development in the A. mississippiensis is the long thin smooth endoplasmic reticulum that cov-
ers the rostral acrosome, which we term the endoplasmic reticular cap (Fig. 2A–C, black arrows).

Towards the termination of the round spermatid stage, the most distal part of the nucleus starts to elongate (Fig. 2D). As the spermatid nucleus stretches, it takes on a cylindrical appearance (Fig. 3A, Nu). The same organelles, mitochondria (Fig. 3A, black arrow) and smooth endoplasmic reticulum (Fig. 3A, white arrowheads; 3B, black arrows), are observed within the cytoplasm of elongating spermatids as are seen within the round spermatid stage. The acrosome begins to envelop and migrate laterally along the apex of the nucleus (Fig. 3A–C). The acrosome is sandwiched between the cell membrane (Fig. 3B, black arrowhead; 3C, white arrow) and the nuclear apex. The acrosome granule is large and basally located (Fig. 3B, white arrow; 3C, black arrowhead), and it appears that dense material from the granule is diffusing out into the acrosome vesicle lumen. There is a prominent and dark staining subacrosome space between the inner acrosome membrane and the apex of the nucleus (Fig. 3C, black arrow). During the elongation phase of spermiogenesis the nucleus contains mostly diffuse euchromatin with only a few pockets of dense staining heterochromatin (Fig. 3B, white arrowhead) randomly located within the nucleoplasm. A transverse view through the early elongate spermatid nucleus reveals that the smooth endoplasmic reticulum is organized in a concentric layer located adjacent to the nuclear membrane (Fig. 3D, white arrows).

As elongation continues, the acrosome vesicle begins to envelop the nucleus by moving caudally along its lateral edges, and the acrosome granule has completely diffused throughout the acrosome lumen (Fig. 4A). This results in flocculent material within the lumen of the acrosome vesicle (Fig. 4A, *). Chromatin condensation has commenced and the chromatin begins to bead in a granular style (Fig. 4A, Nu). As the acrosome shoulders (Fig. 4A white arrow; 4B, *) migrate laterally they appear to squeeze down on the apex of the nucleus resulting in a thin papilla shaped apical head that will continue to thin into the mature rostrum of the nucleus (Fig. 4B). Within the developing rostrum

Fig. 1. Light microscope views of an April seminiferous tubule in the American Alligator testis. (A) Low power view of the seminiferous tubule in transverse section. The tubule has a lumen (L) where sperm will be released after the completion of spermiogenesis. The germinal epithelium is thick (black line) and contains developing germ cells and Sertoli cells (white arrow). Bar = 50 μm. (B) High power view of the germinal epithelium showing generations of developing germ cells. The spermatogonia and spermatocytes (M) are located near the basement membrane (black arrowhead) at the periphery of the seminiferous tubule. The round spermatids (R), which are situated between the meiotic/mitotic cells and the elongating spermatids. The elongating spermatids (E) are located near the apex of the germinal epithelium in close proximity to the lumen (L). Bar = 20 μm. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]
an endonuclear canal starts to form and will house the perforatorium in more mature spermatids (Fig. 4A, white arrowhead; inset, white arrowhead; 4B, black arrow). The subacrosomal space becomes more prominent and is filled with a condensed dark staining granular layer (Fig. 4B, white

Fig. 2. Round spermatids undergoing acrosome development during the early stages of spermiogenesis within the testes of *Alligator mississippiensis*. Common features of the round spermatids include smooth endoplasmic reticulum (SR) and mitochondria (white arrow) in the cytoplasm, and a thin band of smooth endoplasmic reticulum (black arrow) lying apical to the acrosomal vesicle (Av). (A) The acrosomal vesicle lies apical to the nucleus (Nu), but is not yet in contact with the nucleus. Bar = 1 µm. (B) The acrosomal vesicle is in contact with the nucleus (Nu) and the acrosomal granule (white arrowhead) has now formed. Bar = 1 µm. (C) Transport vesicles (black arrowhead) of the Golgi apparatus (black arrow) merging with the acrosomal vesicle. Bar = 0.5 µm. (D) The acrosome vesicle has collapsed and flattened the apical surface of the nucleus (Nu), and the subacrosomal space (black arrowhead) can now be observed. Bar = 1 µm.
Fig. 3. Germ cells immediately after the round spermatid stage when spermatid elongation begins within the testes of *Alligator mississippiensis*. (A) Nucleus (Nu) elongating and capped by the acrosomal vesicle stretching posteriorly over the nucleus. Mitochondria (black arrow) and smooth endoplasmic reticulum (white arrowheads) are common in the cytoplasm. Bar = 1 μm. (B) High magnification of the acrosomal region of the elongating spermatid demonstrating the dense material of the acrosomal granule (white arrow) diffusing within the lumen of the acrosomal vesicle. At this stage the acrosomes is sandwiched between the cell membrane (black arrow) and nuclear apex, and cisternae of smooth endoplasmic reticulum are present cytoplasmically (black arrows). During elongation the nucleus (Nu) is euchromatic with pockets of dense staining chromatin (white arrowhead). Bar = 1 μm. (C) Apex of the nucleus (Nu) highlighting the orientation of the nucleus, cell membrane (white arrow), acrosomal vesicle (Av), and acrosomal granule (black arrowhead) in an early elongating spermatid. Subacrosome space (black arrow). Bar = 0.5 μm. (D) Transverse section through an early elongating spermatid nucleus (Nu) showing the concentric layers of smooth endoplasmic reticulum (white arrow) organized adjacent to the nuclear membrane. The axoneme (black arrowhead) can also be observed abutting the cell membrane, and mitochondria (black arrow) are scattered throughout the early elongating spermatid cytoplasm. Sertoli cell process (Sp). Bar = 1 μm.
At the peak of elongation, the acrosome vesicle envelops the entire nuclear apex and the developing rostrum is triangular in shape (Fig. 5, black arrows). The nucleus has reached its final length of over 25 μm (Fig. 5, black brackets). The endonuclear canal and the enclosed developing perforatorium have increased in length and span the entire immature rostrum, and they extend all the way to the tip of the nucleus (Fig. 5, white arrowheads).

During late elongation the chromatin becomes more condensed and large chromatin granules are closer together and little to no nucleoplasm is observed (Fig. 6A, NU). The microtubules of the manchette (Fig. 6B, black arrows and D, white arrows) become evident on the lateral aspects of the nucleus as the spermatids continue to thin.
Most of the manchette is made up of parallel microtubules (Fig. 6B, black arrows) that can be found surrounding the length of the nucleus (Fig. 6A, white *) caudally to the acrosome shoulders (white *). The endonuclear canal (white arrow) becomes enlarged and the microtubules of the perforatorium (white arrowhead) can be seen within the canal. The subacrosome space (black *) becomes enlarged and is filled with dense granular material. There is a thin clear lucent zone between the granular layer and the inner acrosome membrane (black arrowhead). Bar = 1 µm. (B) Parallel microtubules of the manchette (black arrows) surrounding the entire length of the nucleus (Nu), and the proximal centriole (white arrowhead) lying in the nuclear fossa. Bar = 1 µm. (C) The distal centriole (white arrow), posterior to the proximal centriole (white arrowhead), elongating into the neck axoneme (black arrowhead), while the annular ring (black arrows) migrates away from the nucleus. Bar = 1 µm. (D) Transverse section through a spermatid in late elongation highlighting the orientation of the nucleus (white *), endonuclear lacuna (white arrowhead), manchette (white arrows), mitochondria (black arrows), and Sertoli cell process (Sp). Bar = 1 µm. (E) Mitochondria (white arrowheads) are arranged in a single row between the nucleus and annulus (white arrows). The midpiece of the flagellum is surrounded by Sertoli cell processes forming the flagellar tunnel (black arrows) as the flagellum continues posterior to the annulus (principal piece) and is surrounded by the fibrous sheath (black arrowhead). Bar = 1 µm.

Fig. 6. Spermatids in late elongation/condensation within the testes of Alligator mississippiensis. (A) The chromatin becomes more condensed in the nucleus (Nu) and forms large chromatin granules. Circular microtubules (black arrow) are located around the nucleus just caudal to the acrosome shoulders (white *). The endonuclear canal (white arrow) becomes enlarged and the microtubules of the perforatorium (white arrowhead) can be seen within the canal. The subacrosome space (black *) becomes enlarged and is filled with dense granular material. There is a thin clear lucent zone between the granular layer and the inner acrosome membrane (black arrowhead). Bar = 1 µm. (B) Parallel microtubules of the manchette (black arrows) surrounding the entire length of the nucleus (Nu), and the proximal centriole (white arrowhead) lying in the nuclear fossa. Bar = 1 µm. (C) The distal centriole (white arrow), posterior to the proximal centriole (white arrowhead), elongating into the neck axoneme (black arrowhead), while the annular ring (black arrows) migrates away from the nucleus. Bar = 1 µm. (D) Transverse section through a spermatid in late elongation highlighting the orientation of the nucleus (white *), endonuclear lacuna (white arrowhead), manchette (white arrows), mitochondria (black arrows), and Sertoli cell process (Sp). Bar = 1 µm. (E) Mitochondria (white arrowheads) are arranged in a single row between the nucleus and annulus (white arrows). The midpiece of the flagellum is surrounded by Sertoli cell processes forming the flagellar tunnel (black arrows) as the flagellum continues posterior to the annulus (principal piece) and is surrounded by the fibrous sheath (black arrowhead). Bar = 1 µm.
the nucleus. Mitochondria also migrate (Fig. 6D, black arrow) toward the developing flagellum caudally and move in the direction of the annulus (Fig. 6E, white arrows) and away from the nucleus, and arrange themselves in a single row (Fig. 6E, white arrowhead) in sagittal section. The Sertoli cell processes enveloping late elongate spermatids extend caudally to the midpiece of the flagellum forming the flagellar tunnel (Fig. 6E, black arrowhead). The flagellum continues to elongate caudally past the annulus and becomes surrounded by a surplus of fibrous blocks (Fig. 6E, black arrowhead) creating a fibrous sheath around the principal piece of the flagellum. The axoneme of the midpiece, principal piece, and endpiece is made up of the typical $9 + 2$ arrangement of microtubule doublets.

The final stage of spermiogenesis demonstrates many of the presumed mature structures that will be present within the American Alligator spermatids in the final stage of spermiogenesis within the testes of Alligator mississippiensis. (A)–(G) on the medial sagittal section represent the approximate location where the peripheral transverse sections were taken from. Sagittal section. Proximal centriole (black arrow). Bar = 1 μm. (A) The tip of the acrosome demonstrating the dark staining cortex (black arrow) and lighter staining medulla (M) surrounded by the multilaminar layers of the Sertoli cell membrane (white arrowhead) and smooth endoplasmic reticulum (white arrow; labeled identically in (B)–(D)). (B) The acrosome (black arrow) rests upon a dense subacrosome space (Sa). (C) An epinuclear lucent zone (white arrowhead) rests on the tip of the nuclear rostrum within the subacrosome space (white arrowhead), lying medial to the acrosome (black arrow). (D) Medial to the acrosome (black arrow) and subacrosome space (*) lays the rostrum (white *), in which microtubules of the perforatorium reside within the endonuclear canal (white arrowhead). (E) The body of the nucleus (Nu) is surrounded by parallel microtubules of the manchette (black arrow). (F) Large mitochondria (white arrow), with tubular cristae surrounded by concentric layers of cristae, surround the axoneme and dark staining columns (black arrow). (G) A fibrous sheath (black arrow) internal to the cell membrane (black arrowhead) surrounds the axoneme (white arrow) in the principal piece of the spermatid. (H) The endpiece axoneme (black arrow) lacks a fibrous sheath and is encompassed only by the cell membrane (black arrowhead). Bars = 0.2 μm.
there are intra-acrosomal vesicles that deliver granules (Dehlawi et al., 1992), and within the subacrosomal space (Fig. 7B, Sa), and a thin nuclear rostrum extends into the subacrosomal space (Fig. 7D, white asterisk). The main body of the acrosome has a lighter staining medulla (Fig. 7A,M) and a dark staining thin cortex (Fig. 7A, black arrow). Multilaminar layers of Sertoli cell membrane (Fig. 7A, white arrowhead) are common around the acrosome complex of these late developing spermatids. In these late developing spermatids, a ring of smooth ER is common around the acrosome complex (Fig. 7B–D, white arrow). A small epinuclear lucent zone rests on the tip of the nuclear rostrum (Fig. 7C, white arrowhead) within the subsacrosomal space (Fig. 7C, white asterisk). Within the nuclear rostrum (Fig. 7D, white asterisk) of a mature spermatid, a distinct endonuclear canal (Fig. 7D, white arrowhead) exists and has the enclosed perforatorium. The body of the nucleus is cylindrical and is surrounded by the parallel microtubules of the manchette (Fig. 7E, black arrow). The midpiece has large mitochondria that have tubular cristae internally and concentric layers of membranes externally (Fig. 7F, white arrow) surrounding the axoneme (Fig. 7F, black arrow). The midpiece axoneme lacks a fibrous sheath; however, the microtubule doublets of the midpiece are surrounded by dark staining granular columns (Fig. 7F, black arrow). The principal piece beyond the annular ring does have a fibrous sheath that is seen in cross section (Fig. 7G, black arrow), and the endpiece is easily distinguished from the principal piece, as it lacks a fibrous sheath around its axoneme (Fig. 7H, black arrow).

**DISCUSSION**

Most of the ultrastructure features of spermiogenesis within *Alligator mississippiensis* are similar to those described for other reptilian sauropsids. The early development of the acrosome complex within the *A. mississippiensis* testes is akin to what has been described for other amniote taxa. The single acrosome vesicle forms from transport vesicles delivered from the Golgi apparatus. This is comparable to what has been described for *Sphenodon* (Healy and Jamieson, 1994), squamates (Gribbins et al., 2007, 2009; Rheubert et al., in press), and other archosaurs including birds (Aire, 2007) and crocodilians (Saita et al., 1987; Wang et al., 2008). However, at least one species of agamid lizard was reported to have two acrosome vesicles and granules (Dehlawi et al., 1992), and within *Sphenodon* there are intra-acrosomal vesicles that deliver granules to the exterior of the outer acrosome membrane apically (Healy and Jamieson, 1994). We identified a single unique feature in early acrosome development in *A. mississippiensis*: a thin layer of endoplasmic reticulum forms a cap on top of the developing acrosome vesicle.

A single large acrosome vesicle forms and remains in contact with the inner acrosome membrane throughout sperm development until mid to late elongation when it diffuses uniformly throughout the acrosome lumen. The nucleus of the American alligator has uniform diffuse chromatin with small indices of heterochromatin, which is much different from the intermediate to heavily heterochromatic nuclei of *Sphenodon* (Healy and Jamieson, 1994) and chelonians (Sprando and Russell, 1988; Healy and Jamieson, 1992; Zhang et al., 2007). Conversely, like other crocodilians (Saita et al., 1987; Wang et al., 2008), the condensation of DNA in *Alligator mississippiensis* packs into progressively larger granules until the nucleus contains only homogeneous dark staining DNA, resembling *Sphenodon* (Healy and Jamieson, 1994) and chelonians (Sprando and Russell, 1988; Healy and Jamieson, 1992; Al-Dokhi and Al-Wasel, 2001b; Zhang et al., 2007). In squamates, such as *Agristrodon piscivorus* (Gribbins et al., 2009), *Anolis carolinensis* (Rheubert et al., in press), and *Scincella laterale* (Gribbins et al., 2007), the DNA condenses in a filamentous helical fashion. In *A. mississippiensis*, early to mid elongating spermatids have an adjacent layer of endoplasmic reticulum that surrounds the nucleus, which may be an autapomorphic feature of spermiogenesis in *A. mississippiensis* as this has not been reported in any other taxa, including previously crocodilians (Saita et al., 1987; Wang et al., 2008). The subacrosome space develops early in the round spermatid stage in *A. mississippiensis* and continues to enlarge during elongation, and this space accumulates a thick layer of dark staining granules similar to that described in other reptilian sauropsids (Sprando and Russell, 1988; Aire, 2007; Gribbins et al., 2007, 2009). Also like other reptilian sauropsids (Clark, 1967; Sprando and Russell, 1988; Aire, 2007; Gribbins et al., 2007, 2009), once the acrosome has completed its development and growth, the nuclei of the early elongating spermatids become associated with the cell membrane. This contact with the cell membrane flattens the acrosome vesicle on the surface of the anterior nucleus, which aids in the migration of the acrosomal shoulders laterally over the apical nuclear head.

In *Sphenodon* (Healy and Jamieson, 1994), chelonians (Sprando and Russell, 1988; Healy and Jamieson, 1992; Al-Dokhi and Al-Wasel, 2001a,b, 2002; Zhang et al., 2007), non-passarine birds (Aire, 2007) and other crocodilians (Saita et al., 1987; Wang et al., 2008), at least one endonuclear canal is present that is rod shaped and houses the perforatorium. Within crocodilians and other arch-
Tripepi, 2005). The functions of these spaces are and in many non-passerine birds (Jamieson and Ibarguengoytia and Cussac, 1999). It within the nucleus of A. mississippiensis, the rods extend past the acrosomal region deep into the nuclear body in middle elongating spermatids. There has been some controversy on whether a perforatorium exists within the endonuclear canals of some reptilian sauropsids (Saita et al., 1987), however, in A. mississippiensis, a clearly visible perforatorium (dark staining rods) develops within the endonuclear canal, much like that observed in the Caiman (Saita et al., 1987). In contrast, all squamates studied to date have an extranuclear perforatorium (with no endonuclear canals) located in the subacrosome space within their spermatids and spermatooza (Jamieson, 1995; Gribbins et al., 2007, 2009; Rheubert et al., in press).

In Alligator mississippiensis, we observed a prominent longitudinal manchette associated with the nuclei of late elongating spermatids. Also present, but less numerous, were circular microtubules associated with the nucleus close to the acrosome complex. The manchette has been implicated in the elongation process of the nucleus during spermiogenesis (Russell et al., 1990) and is considered a common structure observed during spermiogenesis in reptilian sauropsids (Ferreira and Dolder, 2002). Many archosaurs have both circular and longitudinal components to their manchettes (Saita et al., 1987; Lin and Jones, 1993; Soley, 1997; Aire, 2007) as do many squamates (Da Cruz-Landim and Da Cruz-Hofing, 1977; Butler and Gabri, 1984; Dehlawai et al., 1992). However, whether both components are needed for elongation is not known and is complicated by the fact that some squamates have only the longitudinal microtubules (Gribbins et al. 2007, 2009), and at least one species of anole (Rheubert et al., in press) lacks a manchette altogether and elongation still occurs normally. There is only a single type of microtubule that makes up the circular and longitudinal manchette in A. mississippiensis, which is different from the Caiman (Saita et al., 1987) where the microtubules of the longitudinal manchette are thicker walled than those found in the circular manchette.

During the late stages of elongation in some chelonians and some squamates, there are lucent zones within the distal bodies of the elongate spermatid nuclei. These clear zones have been called nuclear lacunae or intranuclear tubules (Jamieson et al., 1996; Ibarguengoytia and Cussac, 1999). It is not unusual to see these same types of lacunae within the nucleus of Alligator mississippiensis, and in many non-passerine birds (Jamieson and Tripepi, 2005). The functions of these spaces are still unknown within these developing spermatids. There is also an epinuclear lucent zone present within the A. mississippiensis acrosome complex as seen in many other reptilian taxa (Jamieson, 1995; Ferreria and Dolder, 2002; Gribbins et al., 2007, 2009). The A. mississippiensis flagellum develops similarly to that described in other reptiles to date. The proximal centriole rests within the nuclear fossa of A. mississippiensis spermatids. The axoneme develops in continuity with the distal centriole. There is no prominent neck region and no peritubular dense material around this region in A. mississippiensis. The mitochondria of the midpiece abut the proximal centriole resulting in a very short neck region, which is congruent to what is observed within the Caiman (Saita et al., 1987) and chelonians (Sprando and Russell, 1988; Healy and Jamieson, 1992; Al-Dokhi and Al-Wasel, 2001a; Zhang et al., 2007) the rods extend to the acrosomal region deep into the nuclear body in middle elongating spermatids. There has been some controversy on whether a perforatorium exists within the endonuclear canals of some reptilian sauropsids (Saita et al., 1987), however, in A. mississippiensis, a clearly visible perforatorium (dark staining rods) develops within the endonuclear canal, much like that observed in the Caiman (Saita et al., 1987). In contrast, all squamates studied to date have an extranuclear perforatorium (with no endonuclear canals) located in the subacrosome space within their spermatids and spermatooza (Jamieson, 1995; Gribbins et al., 2007, 2009; Rheubert et al., in press).

In Alligator mississippiensis, we observed a prominent longitudinal manchette associated with the nuclei of late elongating spermatids. Also present, but less numerous, were circular microtubules associated with the nucleus close to the acrosome complex. The manchette has been implicated in the elongation process of the nucleus during spermiogenesis (Russell et al., 1990) and is considered a common structure observed during spermiogenesis in reptilian sauropsids (Ferreira and Dolder, 2002). Many archosaurs have both circular and longitudinal components to their manchettes (Saita et al., 1987; Lin and Jones, 1993; Soley, 1997; Aire, 2007) as do many squamates (Da Cruz-Landim and Da Cruz-Hofing, 1977; Butler and Gabri, 1984; Dehlawai et al., 1992). However, whether both components are needed for elongation is not known and is complicated by the fact that some squamates have only the longitudinal microtubules (Gribbins et al. 2007, 2009), and at least one species of anole (Rheubert et al., in press) lacks a manchette altogether and elongation still occurs normally. There is only a single type of microtubule that makes up the circular and longitudinal manchette in A. mississippiensis, which is different from the Caiman (Saita et al., 1987) where the microtubules of the longitudinal manchette are thicker walled than those found in the circular manchette.

During the late stages of elongation in some chelonians and some squamates, there are lucent zones within the distal bodies of the elongate spermatid nuclei. These clear zones have been called nuclear lacunae or intranuclear tubules (Jamieson et al., 1996; Ibarguengoytia and Cussac, 1999). It is not unusual to see these same types of lacunae within the nucleus of Alligator mississippiensis, and in many non-passerine birds (Jamieson and Tripepi, 2005). The functions of these spaces are still unknown within these developing spermatids. There is also an epinuclear lucent zone present within the A. mississippiensis acrosome complex as seen in many other reptilian taxa (Jamieson, 1995; Ferreria and Dolder, 2002; Gribbins et al., 2007, 2009). The A. mississippiensis flagellum develops similarly to that described in other reptiles to date. The proximal centriole rests within the nuclear fossa of A. mississippiensis spermatids. The axoneme develops in continuity with the distal centriole. There is no prominent neck region and no peritubular dense material around this region in A. mississippiensis. The mitochondria of the midpiece abut the proximal centriole resulting in a very short neck region, which is congruent to what is observed within the Caiman (Saita et al., 1987) and chelonians (Sprando and Russell, 1988; Healy and Jamieson, 1992; Al-Dokhi and Al-Wasel, 2002). The microtubular doublets of the midpiece axoneme are surrounded by a layer of dense staining granular material that is similar to that observed within the midpiece axoneme of Caiman spermatids (Saita et al., 1987). These dense staining materials around the microtubules of the midpiece are equivalent to the striated columns found within mammalian spermatooza (Lindemann, 1996) and most likely aid to reinforce the midpiece axoneme. The mitochondria of the midpiece, at least in the late elongating spermatids of A. mississippiensis, have tubular cristae internally and concentric layers of cristae superficially. This is slightly different from what is seen in the Caiman, which has concentric rings of cristae superficially and an electron-dense center that lacks tubular cristae. There are typically 7-9 concentric rows of mitochondria in a sagittal section of the A. mississippiensis midpiece and 7-8 concentric mitochondria within each row in cross section. These numbers seem to be consistent with what has been observed in other crocodilians (Saita et al., 1987). The concentric layers of cristae are also observed within chelonians (Hess et al., 1991; Healy and Jamieson, 1992) and Sphenodon (Healy and Jamieson, 1994). Since sperm storage is common in the female tract of turtles (Gist and Jones, 1989, Gist and Fischer, 1993, Gist and Congdon, 1998), and has recently been described in A. mississippiensis (Gist et al., 2008; Bagwill et al., 2009), it is possible that these concentric rings of cell membrane may provide energy reserves that aid the sperm's ability to survive long and short periods of time within the female reproductive tract before fertilization. The midpiece terminates at the annulus within A. mississippiensis spermatids as that described for most reptilian sauropsids. Also, like Sphenodon, chelonians, and other archosaurus the fibrous sheath of the principal piece does not penetrate into the midpiece, penetration being a synapomorphy for the squamates (Jamieson, 1995). The endpiece in American alligators is easily distinguished from the principal
piece, as the axoneme is not surround by the fibrous sheath.

Although the characteristics of spermiogenesis within *Alligator mississippiensis* are similar to what has been described in other amniotes, there are four ultrastructural features of the *A. mississippiensis* spermatids that seem to be unique and what has been described in other amniotes, superficially. Alligator *mississippiensis* spermatids also share two major features with *Sphenodon*, chelonians, and other archosaurs: (1) endonuclear canals; (2) the lack of the fibrous sheath within the midpiece. These characteristics have not been reported for Squamata. The importance of such differences and similarities in structure of the rep-tilian spermatids between closely and distantly related species is unknown as there are not sufficient representative data from squamates, archosaurs, and turtles to perform robust phylogenetic analyses using morphological characters from spermiogenesis. It is noteworthy to mention as do Saita et al. (1987) that the crocodilian spermatids, like that of the Caiman, seem to share far more ultrastructural features with those of chelonians and *Sphenodon* than they do with Squamata. Furthermore, Jamieson (2007) has shown that the sperm of paleopnath birds are so similar to those of croco-dilians as to be termed “crocodiloid.” However, most of the similarities between the sperm of che-lonians and those of Sphenodon and crocodilians have been shown in a cladistic analysis to be sym-plesiomorphies of these basal amniote groups (Jamieson and Healy, 1992). Until further mor-phological data sets are produced from spermiogenesis and spermatozoo ultrastructure within all the major groups of reptiles and birds answers to these questions will remain tentative. The ultrastructural study on *A. mississippiensis* spermiogenesis presented here provides baseline data that should be utilized in future evolutionary and histo-pathological investigations.

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