

Seasonal Variation of the Oviduct of the American Alligator, *Alligator mississippiensis* (Reptilia: Crocodylia)

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ABSTRACT The annual oviductal cycle of the American alligator, *Alligator mississippiensis*, is described using light and electron microscopy. Previous work done by Palmer and Guillette ([1992] Biol Reprod 46:39–47) shed some light on the reproductive morphology of the female alligator oviduct; however, their study was limited and did not report details relating to variation across the reproductive season. We recognize six variable regions of the oviduct: infundibulum, tube, isthmus, anterior uterus, posterior uterus, and vagina. Each area shows variation, to some degree, in the histochemistry and ultrastructure of oviductal secretions. Peak secretory activity occurs during the months of May and June, with the greatest variation occurring in the tube and anterior uterus. During the month of May, high densities of neutral carbohydrates and proteins are found within the tubal and anterior uterine glands. The epithelium of the entire oviduct secretes neutral carbohydrates throughout the year, but many regions lack protein secretions, and the posterior uterine glands show little secretory activity of any type throughout the year. After oviposition, secretory activity decreases drastically, and the oviduct resembles that of the premating season. This study also provides evidence to support the homology between alligator and bird oviducts. Sperm were observed in glands at the tubal-isthmus and utero-vaginal junctions in preovulatory, postovulatory and postovipository females. J. Morphol. 270:702–713, 2009. © 2009 Wiley-Liss, Inc.

KEY WORDS: alligator; reproductive, oviduct, sperm storage, morphology

INTRODUCTION

Crocodylians are grouped with both birds and dinosaurs to comprise the Archosauria (Gauthier, 1984, 1986). This grouping has traditionally been based upon morphological evidence (Gauthier et al., 1988; Hutchinson and Gatesy, 2000; Ras-skin-Gutman and Buscalioni, 2001), but molecular data have more recently been used to confirm this relationship (Brochu, 2001; Chang et al., 2002). The 23 extant species of crocodylians occur circum-tropically. Currently, they are divided into three families: Alligatoridae (four genera, eight species), Crocodylidae (four genera, 14 species), and Gavialidae (one genus, one species). Research on the biology of crocodylians is limited, and the most studied species is the American alligator, *Alligator mississippiensis*.

The range of the American alligator spans the southeastern coastal plain from central Texas east to North Carolina, with small populations reaching the southeast corner of Oklahoma and the southern third of Arkansas in the most northern part of their range (Conant and Collins, 1998). Alligators were close to extinction during the 1970s due to overhunting; however, effective management plans were put into place and now the population is well over several million. Louisiana has the highest population at around 1.5 million wild alligators (Louisiana Department of Wildlife and Fisheries, 2007), followed by Florida with around 1 million (Florida Fish and Wildlife Conservation Commission, 2007). Alligators typically inhabit freshwater swamps and marshes, and occasionally are found in brackish water. In the more temperate regions of their range, they create burrows for shelter/hibernation during the cooler months.

Reproductive characteristics of both male and female alligators in southern Louisiana were described by Lance (1989) using specimens mostly obtained from spring to late summer, the period thought to encompass the breeding season. Reports also exist on female cycles of populations in Florida (Guillette et al., 1997; Uribe and Guillette, 2000) and South Carolina (Wilkinson, 1983). Reproduction can occur annually, although some individuals may not reproduce in consecutive years. Courtship usually starts in late April to early May, with mating beginning in mid-late May, and oviposition commences in late June–July (Joanen and McNease, 1980; Lance, 1989). The reproductive season varies, however, between populations across the range distribution

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TABLE 1. Reproductive condition and the time of year specimens were collected^a

Date	SVL (m)	TL (m)	Sperm	Reproductive condition
8 Feb	0.99	2.08		no enlarged follicles
30 Mar	0.91	1.88		no enlarged follicles
6 Apr	0.99	2.08		no enlarged follicles
10 May	0.95	1.96		no enlarged follicles
10 May	Not taken	2.08		no enlarged follicles
10 May	Not taken	1.98		no enlarged follicles
17 May	Not taken	1.8		one 2-cm follicle
31 May	1.13	2.27	TUBAL-ISTHMUS JUNCTION, UTERO-VAGINAL JUNCTION	preovulatory, 36 ovarian follicles ~3 cm
31 May	1.04	2.18	TUBAL-ISTHMUS JUNCTION, UTERO-VAGINAL JUNCTION	postovulatory, 13 eggs left, 11 eggs right
13 Jun	1.04	2.12	UTERO-VAGINAL JUNCTION	postovipository, at nest 40 eggs
13 Jun	0.94	1.96	UTERO-VAGINAL JUNCTION	postovipository, at nest 20 eggs
14 Jun	0.89	1.88		no enlarged follicles
19 Jun	0.82	1.64		one 2-cm follicle
21 Jun	0.99	2.01		preovulatory, 25 ovarian follicles 2–3.5-cm
10 Oct	1.3	2.54		no enlarged follicles

^aSperm present in either the tubal-isthmus junction (TUBAL-ISTHMUS JUNCTION) and the utero-vaginal junction (UTERO-VAGINAL JUNCTION).

as well as with the temperature of the given year (Klaue, 1984; Jacobsen and Kushlan, 1986; Joanen and McNease, 1989).

Female alligators in Florida have been shown to begin vitellogenesis in October, slowing over the winter, and continuing at the beginning of spring (Guillette et al., 1997). Populations from Louisiana have been reported to begin vitellogenesis in March (Joaen and McNease, 1980; Lance, 1989), but sample sizes from fall are small. Less is known about folliculogenesis and fertilization, but immature follicles grow from 1 to 45 mm at the time of ovulation (Lance, 1989).

Seasonal variation within the oviduct of the American alligator was previously described by Palmer and Guillette (1992). They reported only on late-vitellogenic and gravid females from a population in Florida. This study aims to further the knowledge of the histological and ultrastructural variations in the oviduct of the American alligator presented by Palmer and Guillette (1992), as well as to extend their previous study by including female alligators across the entire reproductive season.

MATERIALS AND METHODS

Specimens

Adult female alligators were collected in southern Louisiana from October 2005 to June 2007 at three main locations: Rockefeller Wildlife Refuge, Grand Chenier, LA; Manchac Wildlife Management Area, Manchac, LA; and Vermilion Gator Farm, Abbeville, LA. The alligators were captured by either setting baited hooks or using a four-foot snare. Once collected, the specimens were killed by severing the spinal cord. Specimens were collected under permit number WL-Research-2006-03 issued by Louisiana Department of Wildlife and Fisheries. Protocols were approved by the Institutional Review Board of Southeastern Louisiana University.

Dissection of the reproductive tract was performed, and the left oviduct was injected with and placed in Trump's fixative (2.5% glutaraldehyde and 2.5% formaldehyde in 0.1 M sodium cacodylate buffer at pH 7.4; Electron Microscopy Sciences, Fort

Worth, PA) for ultrastructural investigation, whereas the right oviduct was placed in 10% neutral buffered formalin for histological investigation. The size and reproductive condition of each of the specimens are listed in Table 1. Adobe Photoshop 7.0 was used for editing of all micrographs (Adobe Systems, San Jose, CA).

Light Microscopy

Tissues were taken from the right oviduct at 10 areas: infundibulum, anterior tube, posterior tube, tubal-isthmus junction, isthmus, isthmus-uterine junction, anterior uterus, posterior uterus, uterovaginal junction, and vagina. The tissues were rinsed for 30 min under running water and dehydrated in a graded series of ethanol (70% for 30 min, 95% for 30 min, and two cycles of 100% at 30 min each). After dehydration, the tissues were placed in toluene for two changes at 30 min each. The tissues were subsequently placed in paraffin overnight under vacuum and embedded in paraffin blocks the following morning. Sections 10- μ m thick were cut using a MR3 microtome (Research and Manufacturing Co., Tucson, AZ) and adhered to albuminized slides. Alternating slides were stained with hematoxylin and counterstained with eosin (H&E) for initial histological examination. The remaining slides were alternately stained with either bromphenol blue (BB) for proteins or treated with periodic acid-Schiff's (PAS) for neutral carbohydrates with an alcian blue at pH 2.5 (AB) counterstain for carboxylated glycosaminoglycans. The slides were viewed with a Leica DM2000 microscope (Leica Microsystems, Wetzlar, Germany), and images were acquired with a Leica DFC420 camera (Leica Microsystems, Wetzlar, Germany).

Transmission Electron Microscopy

Tissues from the left oviduct were rinsed with deionized water for 30 min and postfixed with osmium tetroxide for 90 min. After postfixation, the tissues were rinsed again (30 min) and dehydrated in a graded series of ethanol (70%, 95%, and 100%) for 30 min each. The tissues were subsequently placed in a 1:1 ratio of 100% ethanol and propylene oxide for 30 min and cleared in pure propylene oxide for 30 min. The cleared tissues were placed in a 1:1 mixture of propylene oxide and epoxy resin (EmBed 812, Electron Microscopy Sciences, Fort Washington, PA), a 2:1 mixture of resin and propylene oxide, and finally into pure epoxy resin and placed under vacuum for 12 h. The tissues and fresh resin were then placed into molds to be hardened at

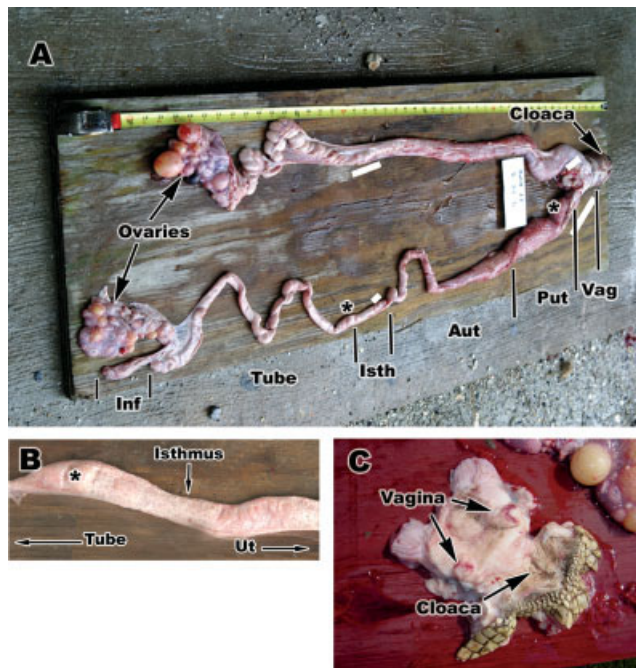


Fig. 1. Gross morphology of a reproductively active oviduct of *Alligator mississippiensis*. (A) Various regions of the oviduct. (B) The transitional region between the tube and uterus, the isthmus. (C) The intramural portion of the vagina via an incision through the center of the cloaca. An asterisk (*) indicates areas where tubular glands possess sperm in reproductively active females. Aut, anterior uterus; Inf, infundibulum; Isth, isthmus; Put, posterior uterus; Ut, uterus; Vag, vagina.

60°C. The tissues were thick-sectioned at 0.5- μ m using a RMC MT7 ultramicrotome (Research and Manufacturing Co., Tucson, AZ) and thin-sectioned at 70 nm with a Leica Reichert Ultracut S ultramicrotome (Leica Microsystems, Wetzlar, Germany) using a DiATOME diamond knife (DiATOME, Biel, Switzerland). The sections were placed on copper grids, stained with uranyl acetate and lead citrate, and viewed with a JEOL JEM 100s transmission electron microscope (TEM) (JEOL USA, Peabody, MA). Images were taken on Kodak film, and negatives were scanned using a Microtek ScanMaker i900 (Microtek International, Carson, CA).

Scanning Electron Microscopy

Through dehydration, procedures followed those for TEM. Tissues were critically point dried using a DCP-1 critical point drier (Denton Vacuum, Moorestown, NJ). The tissues were adhered to stubs and sputter-coated with gold, using a Denton Desk IV XLS (Denton Vacuum, Moorestown, NJ), and viewed on a Philips XL-20 scanning electron microscope (SEM) (Philips Electronics N.V., Eindhoven, the Netherlands).

RESULTS

We distinguish six regions of the American alligator oviduct (cranial to caudal): infundibulum, tube (tuba uterina), isthmus, anterior uterus, posterior uterus, and vagina (see Fig. 1). We do not distinguish a posterior infundibulum (Palmer and Guillelte, 1992; Pérez and Pinilla, 2002), which we

recognize simply as the transition between the infundibulum and the tube.

The entire oviduct is lined by a simple epithelium composed of ciliated cells and nonciliated secretory cells. Throughout the year, the epithelium of the tube and uterus remains active, whereas the production of secretory material in the glands varies, being absent in some regions in certain months (Tables 2, 3). During the inactive periods, neutral carbohydrates and acidic mucoids often are still abundant mostly in the apical portions of the cells. Overall, a peak in the amount of secretory material occurs during the months of May and June and decreases after oviposition to the pre-mating season levels. This trend also corresponds to the peaks of hormone activity reported by Lance (1989) and Guillelte et al. (1997).

This study recognizes two distinct regions of the oviduct which attract aggregates of sperm cells into the tubular glands that occupy the mucosa. These areas are the tubal-isthmus junction and the utero-vaginal junction. Both are at the most posterior end, respectively, of the tube and the uterus, and they possess glands that do not differ from those in adjoining areas except for the presence of sperm. These regions contain sperm within the glands subsequent to the alligator mating season, which suggests that the purpose of these glands is to store sperm. Gist et al. (2008) provide a detailed description of the annual cycle of sperm storage in female *Alligator mississippiensis*.

Infundibulum

The infundibulum is the most anterior portion of the oviduct and lacks tubular glands (Figs. 1A, 2). The tissue layers are very thin, and the infundibulum appears translucent at the gross morphological level. The inner lining consists of many longitudinal folds that create a large amount of surface area. The epithelial cells are squamous to cuboidal and stain basophilic with H&E. The epithelial cells are glandular, and throughout the reproductive cycle variations occur in their secretions (Table 2). During the inactive periods slight amounts of neutral carbohydrates are detected, as well as protein. However, during the reproductive months, these levels increase in density and secretions also stain positively for acidic mucoids. After nesting, the levels of all secretions diminish.

The intercellular canaliculi between the epithelial cells are usually very labyrinthine and relatively wide (Fig. 2A,B), although they narrow in June in females that have oviposited (Fig. 2C). The nuclei are euchromatic with abundant mitochondria surrounding them in inactive months (Fig. 2A) and become more heterochromatic during the reproductive period (Fig. 2B,C). The secretory materials are released into the lumen via an apocrine process (Fig. 2B).

TABLE 2. Abundance of secretions found within the epithelium of the oviduct^a

Date	Inf PAS/AB	Inf BB	Tube PAS/AB	Tube BB	Isth PAS/AB	Isth BB	Aut PAS/AB	Aut BB	Put PAS/AB	Put BB	Vag PAS/AB	Vag BB
8 Feb	0/-	0	+/+	0	0/-	0	+0	0	+0	0	-/-	0
30 Mar	0/0	0	-/+	0	-/-	0	+0	0	0/+	0	N/A	N/A
6 Apr	0/0	0	0/+	0	0/-	0	+0	--	-/-	-	N/A	N/A
10 May	0/0	-	-/-	--	+/+	0	+0	--	0/0	--	N/A	N/A
10 May	0/-	0	+/+	0	-/+	0	+/-	0	+/-	0	N/A	N/A
10 May	-/0	0	+/+	0	0/0	0	+/+	0	+/+	0	N/A	N/A
17 May	-/-	0	-/-	0	-/+	0	-/-	0	-/0	0	N/A	N/A
17 May	-/0	0	+/+	0	-/-	0	+/+	0	+/+	0	N/A	N/A
31 May	+0	+	+/+	0	+0	-	+/+	0	+/-	-	+0	-
31 May	+0	-	+/+	0	+/+	0	+/+	0	+/-	-	-/+	-
13 Jun	-/0	0	+/+	0	+/+	0	+/+	0	+/+	0	-/-	+
13 Jun	-/-	0	+/+	0	+/+	-	+/+	0	+/+	--	+/+	+
14 Jun	-/-	-	+/+	0	+/+	0	+/+	0	+0	0	N/A	N/A
19 Jun	-/0	-	+/+	0	-/+	-	+/+	-	-/-	0	N/A	N/A
21 Jun	-/-	-	+/+	0	-/-	-	+/+	0	-/-	-	N/A	N/A
10 Oct	-/-	0	+/+	0	N/A	N/A	+/+	0	+/-	0	-/0	0

^aAut, anterior uterus; Inf, infundibulum; Isth, isthmus; Put, posterior uterus; Tube, tube; Vag, vagina. 0, no secretion; -, scant secretion; --, very scant secretion; +, abundant secretion; ++, very abundant secretion; N/A, tissue not available; BB, bromphenol blue (proteins); PAS/AB, periodic acid-Schiff/alcian blue (neutral carbohydrates/glycosaminoglycans).

SEM shows the relationships between ciliated and secretory cells (Fig. 2D). The quantity of ciliated cells increases during inactive months.

Tube

The tube is just posterior to the infundibulum and is highly convoluted when the mesentery is intact, but flat once separated. The tube is involved in the synthesis of albumin (Lance, 1989). The tube contains secretory products in the epithelium lining the lumen as well as in compound tubular glands (Figs. 3, 4), and the products of these two secretory areas differ (Tables 2, 3). The columnar epithelium lining the lumen of the tube is usually PAS-positive, AB-positive, and BB-negative whereas the tubal glands are PAS-positive, AB-negative, and BB-positive (Fig. 3A,B). SEM shows that secretory activity continues throughout the reproductive season, although more secretory material is evident in recently mated specimens (Fig. 3C) than one sacrificed after oviposition (Fig. 3D).

Secretory material is heterogeneously electron-dense in the tubal lining of recently mated females (Fig. 4A) and homogeneous in the tubal glands (Fig. 4B). In females collected after oviposition, secretory vacuoles appear heterogeneous in both the tubal lining (Fig. 4C) and tubal glands (Fig. 4D). The intercellular canaliculi are narrow and have fewer interdigitations before oviposition (Fig. 4A,B) than after (Fig. 4C,D). The abundance of secretory material during the breeding season obscures the presence of other cellular organelles.

Isthmus

The isthmus is the transition area between the tube and the uterus (Fig. 1B). The isthmus con-

tains ciliated and secretory columnar cells but lacks tubular glands. On the gross morphological scale, it appears very flat and somewhat translucent, averaging around 2.5 cm in length in reproductively active females. Before mating, the apical portion of the epithelium is full of AB-positive material, which becomes absent during peak mating in May when the entire epithelium stains highly PAS-positive (Table 2). After oviposition, the apical portions of the cells secrete both neutral carbohydrates (PAS-positive) and acidic mucosubstances (AB-positive). Protein secretion is absent or scant throughout the year (Table 2).

Ultrastructurally, secretory vacuoles of medium density are present in the apical portions of cells

TABLE 3. Abundance of secretions found within the glands of the oviduct^a

Date	Tgl PAS/AB	Tgl BB	Augl PAS/AB	Aug BB	Pugl PAS/AB	Pug BB
8 Feb	-/0	+	-/-	-	0/0	0
30 Mar	+/-	+	-/0	0	-/0	0
6 Apr	-/0	-	0/0	0	0/0	0
10 May	-/0	-	0/0	0	0/0	0
10 May	+/-	--	--/-	0	--/0	0
10 May	-/-	0	-/-	0	-/-	0
17 May	-/-	0	-/0	0	-/-	0
17 May	+/+0	++	-/-	+	-/0	0
31 May	+/+0	++	-/+	++	+/-	0
31 May	+/+0	++	-/+	++	+/-	0
13 Jun	+/+0	+	0/0	+	-/0	0
13 Jun	+/+0	+	-/-	+	-/0	0
14 Jun	+/+0	+	0/0	-	0/0	0
19 Jun	+/+0	+	-/-	+	-/0	0
21 Jun	+/+0	++	+/-	++	-/-	-
10 Oct	+/+0	0	-/0	0	-/0	0

^aAugl, anterior uterine gland; Pugl, posterior uterine gland; Tgl, tubal gland BB, bromphenol blue; PAS/AB, periodic acid-Schiff/alcian blue (neutral carbohydrates/glycosaminoglycans); 0, no secretion; -, scant secretion; --, very scant secretion; +, abundant secretion; ++, very abundant secretion.

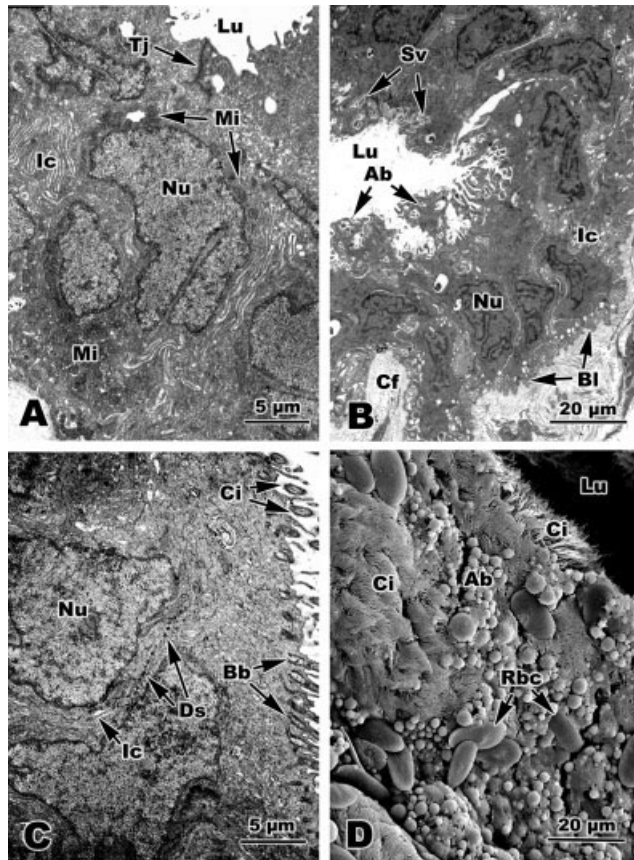


Fig. 2. The infundibulum of *Alligator mississippiensis*. (A) Transmission electron micrograph shows the labyrinthine intercellular canaliculi and the abundant mitochondria in the basal portion of the cell of a reproductively inactive female collected 8 February. (B) Transmission electron micrograph depicting the apocrine secretions released from the epithelium and the wide intercellular canaliculi of a postovulatory female collected 31 May. (C) Transmission electron micrograph showing the ciliated cells and narrow intercellular canaliculi of postovipository female collected 13 June. (D) Scanning electron micrograph of a preovulatory female collected 31 May illustrating the association between secretory and ciliated epithelium. Ab, apocrine blebs; Bb, basal bodies; Bl, basal lamina; Cf, collagen fibers; Ci, cilia; Ds, desmosomes; Ic, intercellular canaliculi; Lu, lumen; Mi, mitochondria; Nu, nucleus; Rbc, red blood cell; Sv, secretory vacuoles; Tj, tight junction.

in preovipository females (Fig. 5A,B), and secretions are not as abundant as in the tubal lining. In females collected after oviposition, the secretory material is scant and secretory vacuoles are electron-lucent (Fig. 5C). In a June postovipository female, electron-lucent secretory vacuoles are still present (Fig. 6C). Intercellular canaliculi are distinct in active females from May (Fig. 5A) whereas in June these cell junctions narrow and are difficult to distinguish (Fig. 5C). SEM confirms that ciliated cells are more numerous than secretory cells, even in recently mated females (Fig. 5D).

Anterior Uterus

The anterior uterus is another highly glandular area with branched tubular endometrial glands. This area starts the formation of the eggshell membranes (Buhi et al., 1999). As in the tube, the epithelial cells of the anterior uterine lining differ in secretory activity from the anterior uterine glands (Fig. 6A,B; Tables 2, 3). The epithelial lining of the anterior uterus consists of columnar cells with basal nuclei; these cells are basophilic throughout the entire year. The epithelium produces PAS+ material during the active months, which is present throughout the entire cell (Fig. 6A). During the rest of the year, this material is still present, but is located only in the apical portion of the cells. The epithelium lining the anterior uterus is AB-negative and BB-negative (Fig. 6A,B). In contrast, the anterior uterine glands are

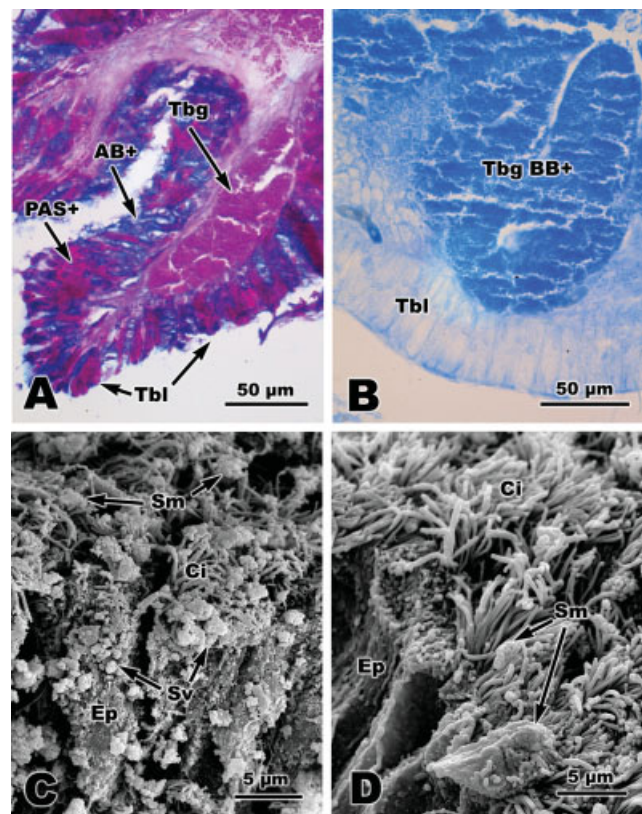


Fig. 3. The tube of *Alligator mississippiensis*. (A) Light micrograph of a postovulatory female collected 31 May treated with PAS for neutral carbohydrates and counterstained with AB for glycosaminoglycans. (B) Same female used in A, stained with BB for proteins. (C) Scanning electron micrograph through the tubal lining of a preovulatory female collected 31 May. (D) Scanning electron micrograph through the tubal lining of a postovipository female collected 13 June. AB+, alcian blue-positive; BB+, bromphenol blue-positive; Ci, cilia; Ep, epithelium; PAS+, periodic acid/Schiff's reagent-positive; Sm, secretory material; Sv, secretory vacuoles; Tbg, tubal glands.

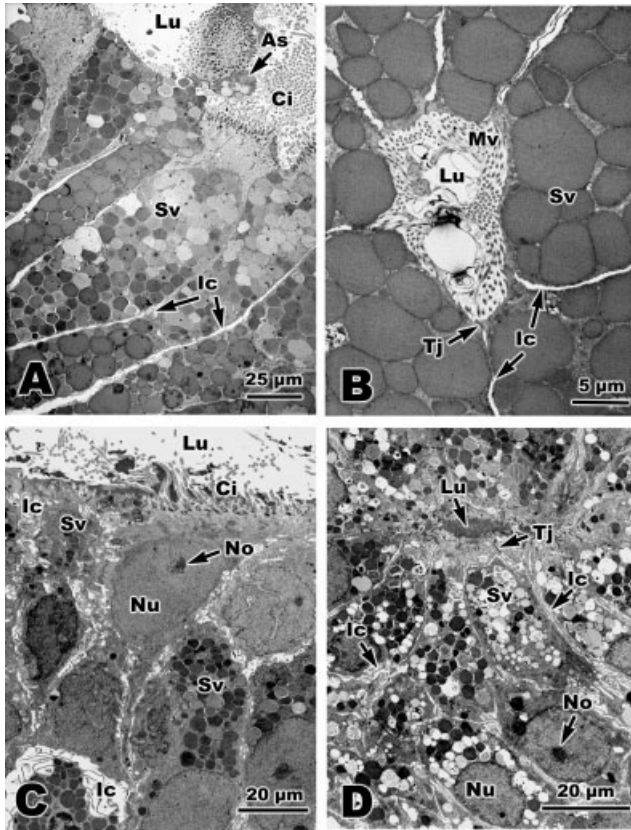


Fig. 4. The tube of *Alligator mississippiensis*. TEM. (A) Tubal lining epithelium of a preovulatory female collected 31 May showing abundant heterogeneous secretory vacuoles and apocrine secretions of the epithelium. (B) Tubal gland of the female used in A, illustrating large homogeneous secretory vacuoles. (C) Tubal lining of a postovipository female collected 13 June showing fewer secretory vacuoles than the May specimen. (D) Heterogeneous secretory material in the tubal glands of the June female used in C. As, apocrine secretion; Ci, cilia; Ic, intercellular canaliculi; Lu, lumen; Mi, mitochondria; Mv, microvilli; No, nucleolus; Nu, nucleus; Sv, secretory vacuole.

strongly BB-positive (Fig. 6B) and show mixed reactions to carbohydrates. In recently mated specimens, much of the epithelium is lightly PAS-positive but the apical cytoplasm of some glands can stain intensely AB-positive (Fig. 6A). SEM reveals the abundance of secretory material throughout the reproductive season in active females (Fig. 6C,D). The clusters of secretory material on the surface of the uterine lining, which are similar to secretory vacuoles observed in the cytoplasm, indicate that an apocrine process is also involved in release of material from this portion of the oviduct.

Before the mating season and in early May, the epithelial lining of the anterior uterus consists of tall columnar cells with dark cytoplasm and little secretory material. Once the mating season begins later in May, the amount of secretory material greatly increases and light cells appear (Fig. 7A).

The light cells, with less electron-dense cytoplasm than dark cells, can contain secretory vacuoles of various sizes and densities (Fig. 7A). The dark cells, however, seem to contain only small vacuoles, which like the cytoplasm, are uniformly electron-dense (Fig. 7A). The anterior uterine glands contain only dark cells, and the secretory vacuoles are quite heterogeneous in both size and density (Fig. 7B).

In postovipository females examined from June, the light cells are no longer observed in the uterine lining, and the dark cells are still filled with small secretory vacuoles, which are so numerous, that they distend the shape of nuclei in the cells (Fig. 7C). Occasional apical cells are observed with disorganized cytoplasm, and we propose that these are cells that have released their product through apocrine processes and are undergoing apoptosis.

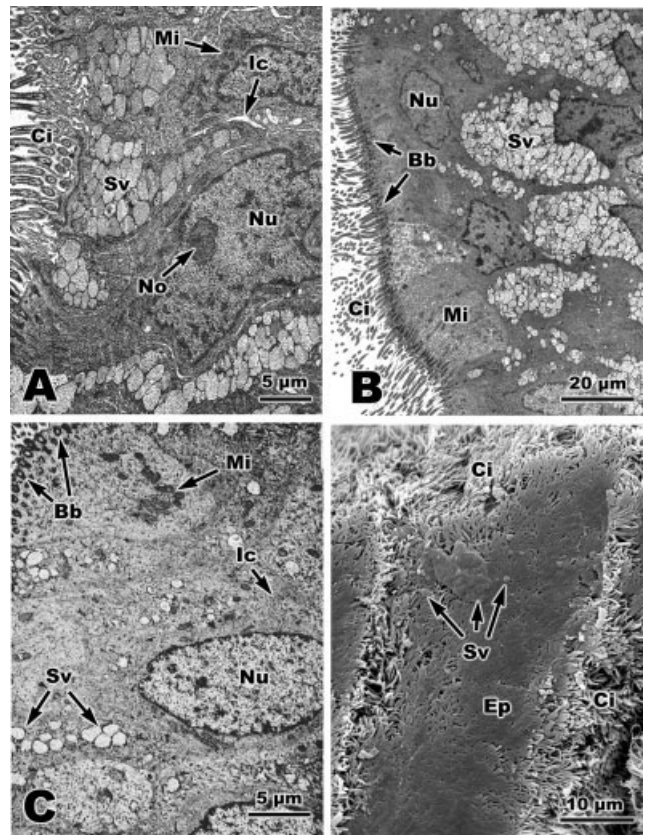


Fig. 5. The isthmus of *Alligator mississippiensis*. (A) Transmission electron micrograph depicting the alternating ciliated and microvillus-secretory cells of the epithelium lining the isthmus of a preovulatory female collected 31 March. (B) Transmission electron micrograph showing abundant ciliated cells and secretory cells with supranuclear electron-lucent secretory material in a preovulatory female collected 21 June. (C) Transmission electron micrograph of a postovipository female collected 13 June showing a decrease in secretory material. (D) Scanning electron micrograph of a postovipository female collected 31 May. Bb, basal bodies; Ci, cilia; Ep, epithelium; Ic, intercellular canaliculi; Mi, mitochondria; Mv, microvilli; No, nucleolus; Nu, nucleus; Sv, secretory vacuoles.

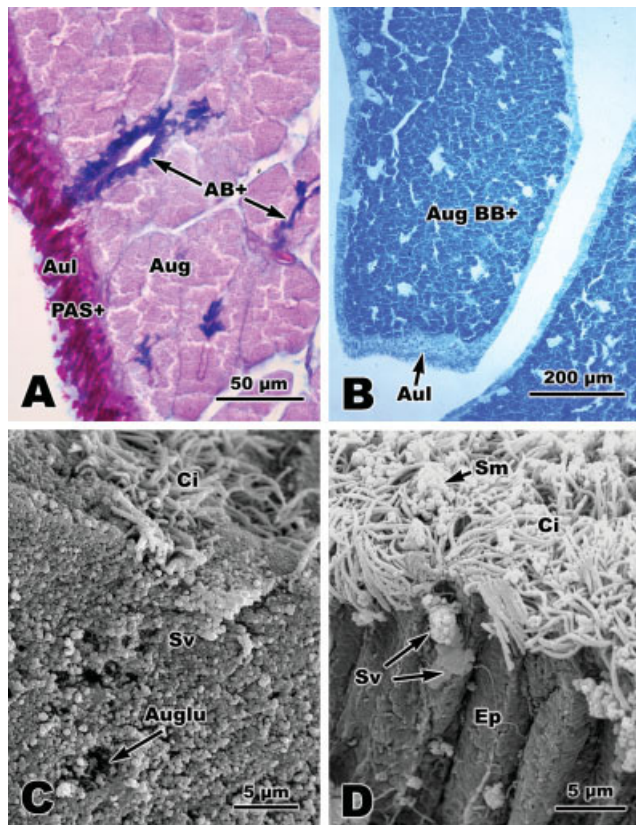


Fig. 6. The anterior uterus of *Alligator mississippiensis*. (A) Light micrograph of the anterior uterine lining of a postovulatory female collected 31 May treated with PAS for neutral carbohydrates and counterstained with AB for glycosaminoglycans. (B) Same female used in A, stained with BB for proteins. (C) Scanning electron micrograph of the anterior uterine epithelium of the female used in A showing abundance of secretory vacuoles. (D) Scanning electron micrograph of the anterior uterine lining of a postovipository female collected 13 June. AB+, alcian blue-positive; Aug, anterior uterine gland; Auglu, anterior uterine gland lumen; Aul, anterior uterine lining; BB+, bromphenol blue-positive; Ci, cilia; Ep, epithelium; PAS+, periodic acid/Schiff's reagent-positive; Sm, secretory material; Sv, secretory vacuoles.

In the anterior uterine glands of female after oviposition, secretory vacuoles of various sizes and densities are still abundant (Fig. 7D). The lucidity of secretory material in lumen indicates that transformation of the products has occurred during the passage of eggs.

Posterior Uterus

The epithelial lining and tubular glands of the posterior uterus differ in secretory activity from the anterior uterus (Figs. 8, 9; Tables 2, 3). This region is presumably involved in secreting the calcareous material of the eggshell (Palmer and Guille, 1992). We did not detect, however, a high degree of secretory activity with ultrastructure

examination (see Fig. 8), although histochemically carbohydrate production was noted (Fig. 9A).

As in the anterior uterus, both electron-light and electron-dense cells are present in the posterior uterine lining but absent in the glands (see Fig. 8). In contrast to the anterior uterus, however, dark cells persist in the posterior uterine lining after oviposition (Fig. 8A,C). Only one type of secretory vacuole with medium density is present in the lining of the posterior uterus, and these are restricted to the dark cells (Fig. 8A,C). The epithelial cells of the posterior uterine have barely distinguishable intercellular canaliculi (Fig. 8A,C).

The posterior uterine glands of recently mated females have wide lumina, wide and interdigitating intercellular canaliculi, and numerous infranuclear mitochondria (Fig. 8B). They are also charac-

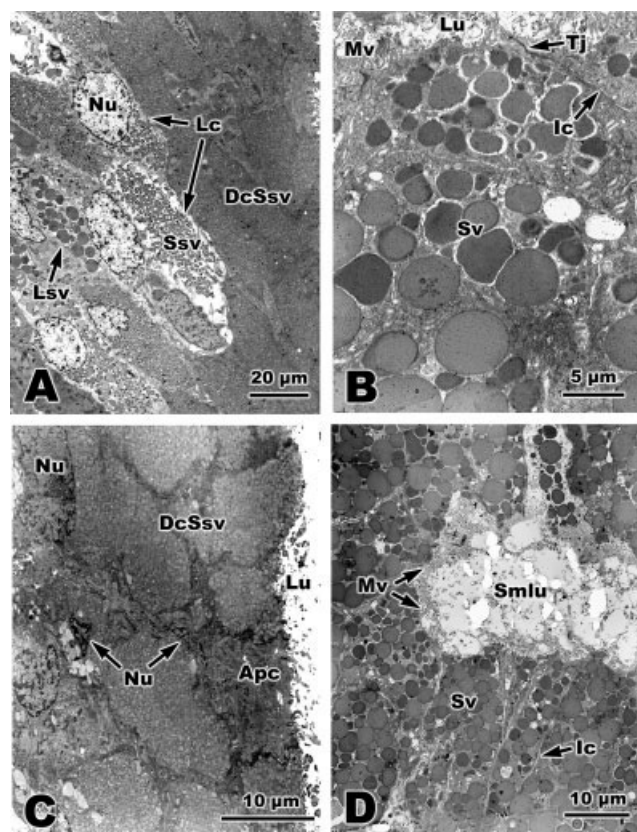


Fig. 7. The anterior uterus of *Alligator mississippiensis*. TEM. (A) The epithelium of a May postovulatory female showing light cells, dark cells, and secretory vacuoles of various sizes. (B) Tubular gland cytoplasm of the May female used in A showing large, heterogeneous secretory vacuoles. (C) Epithelium of a June postovulatory female with no light cells and abundant secretory vacuoles throughout the cells. (D) Tubular gland cytoplasm of the June female used in C with a large lumen and abundant secretory material. Apc, cell undergoing apoptosis; DcSsv, dark cells with small secretory vacuoles; Ic, intercellular canaliculi; Lc, light cells; Lg, gland lumen; Lsv, large secretory vacuoles; Lu, lumen; Mi, mitochondria; Mv, microvilli; Nu, nucleus; Smlu, secretory material in the lumen; Sv, secretory vacuole; Tj, tight junction.

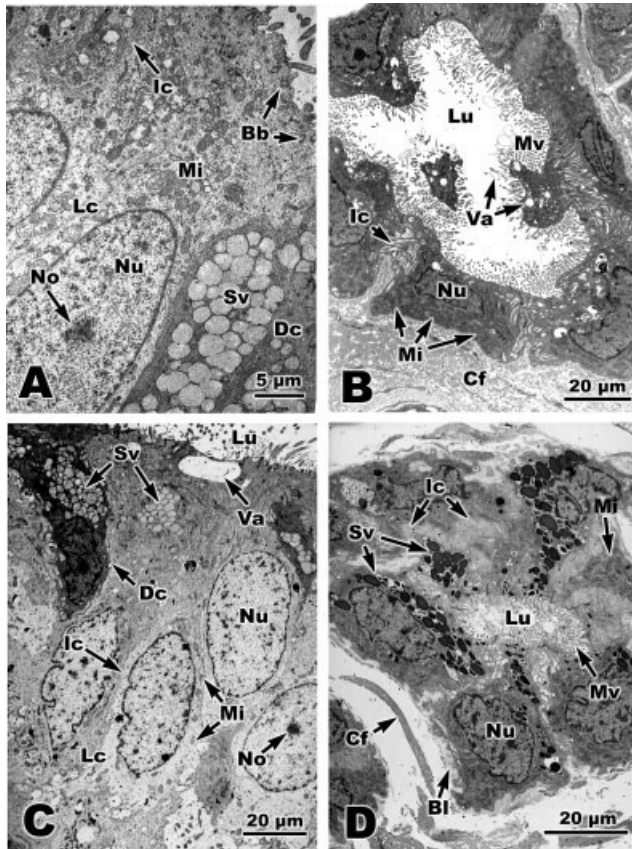


Fig. 8. The posterior uterus of *Alligator mississippiensis*. TEM (A) Uterine lining of a preovulatory female collected 31 May showing abundant supranuclear mitochondria in light cells and secretory material present in dark cells. (B) Uterine gland of the female used in A illustrating the large lumen, abundant mitochondria in the basal portion of the cells, electron-lucent vacuoles, and wide, labyrinthine intercellular canaliculi. (C) Epithelial lining of the posterior uterus of a female collected 13 June following oviposition showing several light cells and scant secretory material in the apical portions of dark cells. (D) Uterine gland of the female used in C showing a small lumen, some electron-dense secretory vacuoles, and wide, labyrinthine intercellular canaliculi. Bb, basal bodies; Bl, basal lamina; Cf, collagen fibers; Ic, intercellular canaliculi; Lu, lumen; Mi, mitochondria; Mv, microvilli; No, nucleolus; Nu, nucleus; Sv, secretory vacuoles; Va, vacuoles.

terized by numerous small, clear vacuoles (Fig. 8B). Posterior uterine glands of postovipository females are similar, but the females examined had electron-dense vacuoles of various sizes (Fig. 8D).

The lining of the posterior uterus stains PAS-positive with occasional AB-positive reactions throughout the year, but the posterior uterine glands are PAS-positive primarily during the mating season (Fig. 9A). Some AB-positive reactions also occur around the luminal border at this time (Fig. 9A). The lining of the posterior uterus stains slightly positive for proteins before the mating season begins, with an increase in protein density during

May (Fig. 9B, Table 2), but the posterior uterine glands rarely react with BB (Fig. 9B; Table 3).

Uterovaginal Junction

The uterovaginal junction possesses a few uterine glands similar to those in the most posterior region of the uterus. Groups of sperm are found in this region from some females collected in May and June, including a preovulatory female (Fig. 9C), a postovulatory female, and a postovipository female (Fig. 9D). After oviposition, however, the amount of sperm is scant. The lumina of glands containing sperm are narrower than those of the posterior uterus, and sperm cells are in close proximity to the apical cytoplasm of gland cells (Fig. 10A,B). With SEM, sperm are illustrated singly in the lumen of the utero-vaginal junction (Fig. 10C).

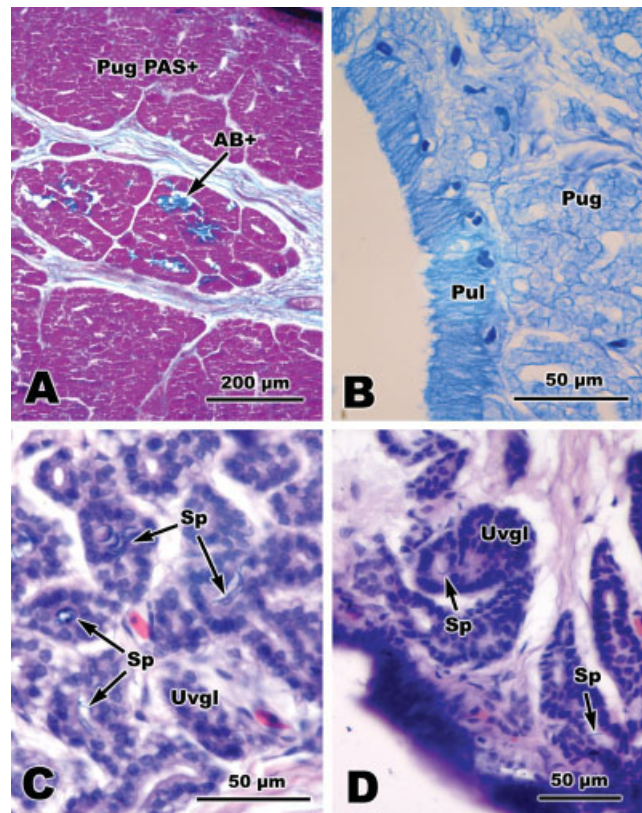


Fig. 9. The posterior uterus (A, B) and uterovaginal junction (C, D) of *Alligator mississippiensis*. LM. (A) Posterior uterine lining of a postovulatory female collected 31 May treated with PAS for neutral carbohydrates and AB for glycosaminoglycans. (B) Same female used in A, stained with bromphenol blue for proteins. (C) Uterovaginal junction of the same female used in A, B, stained with hematoxylin/eosin (H&E), showing numerous sperm in the uterine glands. (D) Uterovaginal junction of a postovipository female collected 13 June stained with H&E, showing single sperm in several uterine glands. AB+, alcian blue positive; PAS+, periodic acid/Schiff's reagent positive; Pug, posterior uterine gland; Pul, posterior uterine lining; Sp, sperm; Uvgl, utero-vaginal gland.

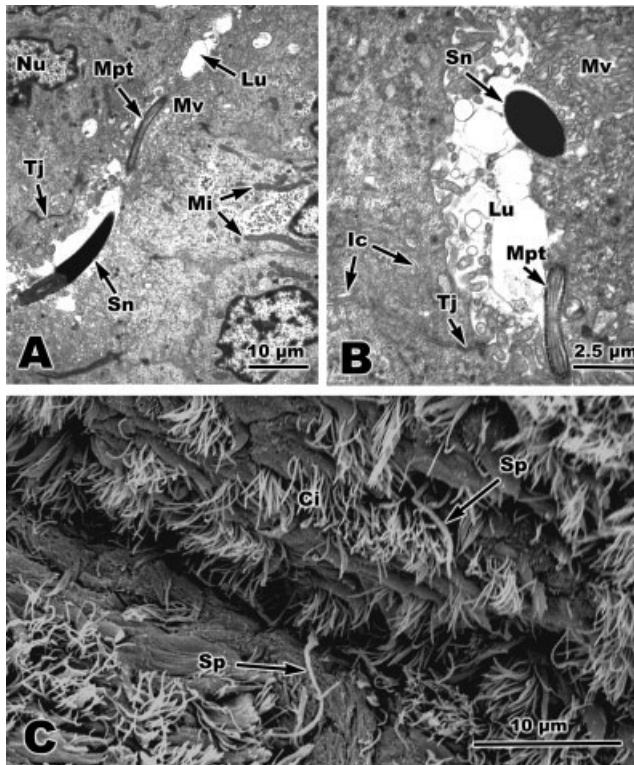


Fig. 10. The uterovaginal junction of a preovulatory *Alligator mississippiensis* collected 31 May. (A) Transmission micrograph showing a sperm with the head pointed toward the opening of a uterine gland into the oviductal lumen. Note the elongate mitochondria. (B) Transmission micrograph illustrating sperm in the lumen of a uterine gland. (C) Scanning micrograph illustrating several sperm in the uterovaginal junction lumen. Ci, cilia; Ic, intercellular canaliculi; Lu, lumen; Mi, mitochondria; Mpt, middle piece of sperm tail; Mv, microvilli; Nu, nucleus; Sn, sperm nucleus; Sp, sperm; Tj, tight junction.

Vagina

The extramural portion of the vagina is a very short region of the oviduct extending only 1–1.5 cm anterior to the cloaca (Fig. 1C). The intramural portion runs 3.5–5.5 cm along the cloaca wall. In our first samples, the shortness of the vagina was not realized, and we failed to include vaginal tissue while excising the oviducts. The vagina lacks tubular glands, but the luminal epithelial lining of the vagina is glandular. During the reproductive season, both PAS+ and BB+ secretory materials are present in scant amounts throughout the vagina (Table 2). Ultrastructurally, the vaginal epithelium seems more complex than what is revealed at the light microscopy (LM) level (Gist et al., 2008). The epithelium has three different types of epithelial cells: ciliated, microvillus secretory, and basal secretory cells (Fig. 11A). The basal cells make the vaginal epithelium appear pseudostriated in areas, but this is not a consistent feature of the lining. Secretory vacuoles are not

numerous but are homogeneously electron-dense and found around the entire nucleus (Fig. 11A). In recently mated females, some of the microvillus cells contain lysosomes (Fig. 11B). A marked decrease in the number of secretory vacuoles occurs after oviposition (Fig. 11C). SEM illustrates that ciliated epithelium dominates the lining of the vagina during the reproductive season (Fig. 11D,E).

DISCUSSION

The results of this study on the alligator oviduct generally coincide with those of Palmer and Guillette (1992) and demonstrate that the oviduct of the alligator is very similar to that of the domestic fowl. The avian oviduct consists of six regions: infundibulum, magnum, isthmus, tubular shell gland, shell gland pouch, and vagina (Solomon,

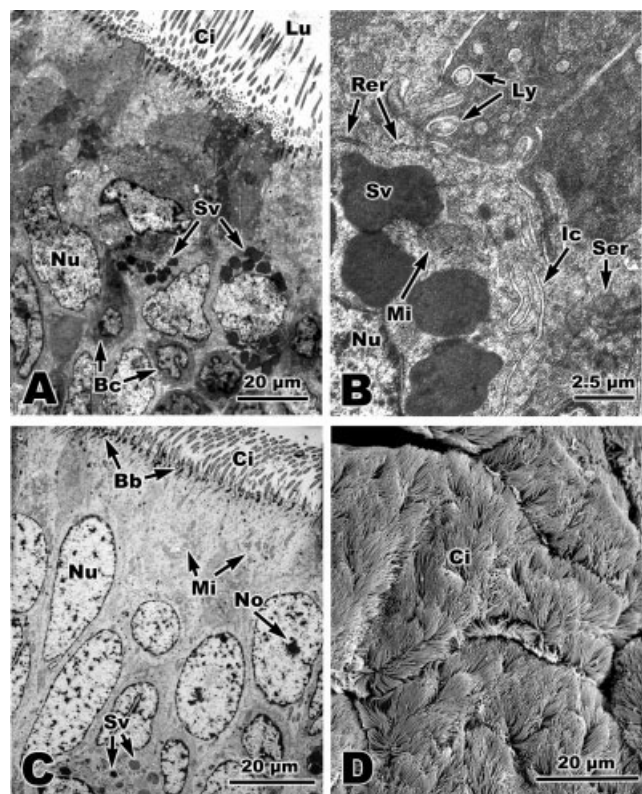


Fig. 11. The vagina of *Alligator mississippiensis*. (A) Transmission micrograph showing ciliated epithelium with several secretory cells in a postovulatory female collected 13 May. (B) Transmission micrograph showing the epithelium with lysosomes and endoplasmic reticulum from the same female as A. (C) Transmission micrograph of the epithelium with spottily heterochromatic nuclei in a postovipository female collected 13 June. (D) Scanning micrograph depicting the ciliated epithelium of the female used in A. Bb, basal bodies; Ci, cilia; Ic, intercellular canaliculi; Ly, lysosomes; Mi, mitochondria; No, nucleolus; Nu, nucleus; Rer, rough endoplasmic reticulum; Sv, secretory vacuole.

1983). Crocodilians have the primitive archosaurian trait of possessing paired oviducts and ovulating eggs through each simultaneously (Lance, 1989; Varricchio et al., 1997; Sato et al., 2005), whereas most birds only develop one oviduct. However, birds also share traits with primitive archosaurs in that only one egg at a time is ovulated and shelled (Solomon, 1983; Varricchio et al., 1997; Sato et al., 2005).

Buhi et al. (1999) found that a large number of proteins are synthesized *de novo* in the alligator oviduct, and these proteins have different biosynthetic activity correlated with location in the oviduct and reproductive status of the female. They suggested that the distribution of proteins synthesized and released support the proposal by Palmer and Guillette (1992) that the alligator oviduct is an intermediate form evolutionarily between birds and ancestral oviparous amniotes.

The magnum of the bird and tube of the alligator are the egg-white protein secreting regions of the oviduct, synthesizing albumen (Asmundson and Baker, 1940; Buhi et al., 1999). Some proteins incorporated into alligator egg albumin appear to be different from those of other species (Buhi et al., 1999). The alligator tube, like the avian magnum, is a highly glandular regions that, during the active times, contains many large secretory granules that are uniformly electron-dense. In the most posterior region of this area (tubal-isthmus junction), alligators possess a sperm storage site, considered the secondary site (Gist et al., 2008). Birds also possess a secondary site; however, it is located in the posterior infundibulum. The primary site for sperm storage in both alligators and birds is the uterovaginal region, also called the shell gland-vaginal region in birds (Solomon, 1983; Baskett et al., 1994).

Both birds and alligators store sperm in the most posterior portions of regions possessing tubular glands. In turtles, sperm are also stored in the most posterior portion of the albumen-secreting region. In several species of chelonians, however, uterine gland sperm storage has also been identified (Gist and Congdon, 1998). The sperm stored in the tubal-isthmus junction are likely the first to exit the glands and are responsible for fertilizing the descending ova. This is supported by the large numbers of sperm present before ovulation followed by scant amounts later. In birds, the infundibulum is the site of fertilization (Bohr et al., 1964), and this is probably true for alligators as well.

High amounts of protein (BB-positive material) were expected in the tube during the reproductively active months because albumen is one of the main egg yolk components (Buhi et al., 1999). The secretions of tubular glands stain positively for proteins, but the epithelial lining of the tube does not, staining instead for neutral and acidic carbohydrates. The secretory products of the lining may

be serving as energy reserves at this time as well as providing other egg yolk components, rather than egg protein production.

Unlike other reptiles where the egg shell fibers and the calcareous outer shell are secreted simultaneously throughout the entire uterus (Palmer and Guillette, 1988; Girling et al., 1998), in both birds and crocodilians the uterus is divided into separate regions for egg shell formation (Solomon et al., 1975; Palmer and Guillette, 1992). The isthmus and shell gland pouch of the avian oviduct are similar to the uterus of the alligator in which they secrete the egg shell fibers and the calcareous outer covering (Solomon et al., 1975). In the anterior portions of the alligator uterus, the egg shell fibers are produced, whereas calcium is deposited in the posterior regions (Palmer and Guillette, 1992). The calcareous outer egg shell is secreted in the shell gland of the bird oviduct (Solomon et al., 1975).

The lack of secretory product found in the tubular glands of the posterior uterus of the alligator may be due in part to the limited histochemical procedures performed. This study tested only the presence of neutral carbohydrates (PAS), acidic mucoids (AB), and proteins (BB) and did not test for the presence of calcium secretions. In the bird oviduct, lipid secretion also occurs in the homologous egg shell gland (Solomon et al., 1975). We did not test for lipids, but lipid droplets were not observed during ultrastructural examination of this region.

The vaginae of birds and alligators are both secretory regions that lack tubular glands. The thick musculature in the posterior end of the oviduct (uterus and vagina) aids in the deposition of eggs (Jones and Guillette, 1982). The occurrence of lysosomes is interesting, as these organelles are important for microbial defense, and the vagina is exposed to any bacteria, etc., that enter the cloaca. Buhi et al. (1999) suggested that the alligator oviduct is part of the secretory immune system.

The only comprehensive study on oviductal morphology of another alligatorid was done by Pérez and Pinilla (2002) on the tropical species *Caiman crocodilus fuscus*. In this study, they distinguish seven separate regions similar to Palmer and Guillette (1992): anterior infundibulum, posterior infundibulum, tube, isthmus, anterior uterus, posterior uterus, and vagina. This study does not recognize the posterior infundibulum as a distinct region; rather, it is designated as the junction between the infundibulum and the tube.

This study has found noticeable differences between *Alligator mississippiensis* and *Caiman crocodilus fuscus*. Pérez and Pinilla (2002) reported that the tubal glands contain abundant eosinophilic secretory granules, which we observed in *A. mississippiensis*, but they found no PAS-positive and AB-positive reactions. In our study, the

tubal glands in reproductively active females were highly PAS-positive, and the epithelial lining of the tube was AB-positive as well. Also, Pérez and Pinilla (2002) stated that sperm storage is occurring in the vaginal folds of the anterior vagina of *C. c. fuscus*, whereas alligators store sperm in the tubal-isthmus junction and the utero-vaginal junction regions of the oviduct. Pérez and Pinilla (2002) only found sperm in a female collected early in the mating season, which suggests that perhaps the female had recently mated, and the sperm did not have a chance to migrate into the storage regions. It is also quite possible that because the areas of storage are very short regions, Pérez and Pinilla (2002) may not have sampled the tissues of the uterovaginal and tubal-isthmus junctions where sperm may have been residing. Further studies are needed to determine if *C. c. fuscus* is actually storing sperm among the vaginal folds.

We collected numerous females that were not in reproductive condition. The first year of specimen collection (2006) was preceded by hurricanes Katrina and Rita, which were then followed by a several month drought at the Rockefeller Wildlife Refuge collection site. The drought caused high salinities in the marshes of the refuge, having double the concentration of sea water. Unfortunately, this drought drastically affected the alligator population and the reproductive season of 2006 causing a 100% decrease in the number of nests found on the refuge (zero were found as opposed to several hundred in normal years). Statewide, the number of nests dropped to about half of that from the previous year (41,392 in 2005 and 20,387 in 2006). Many of the alligators caught, which were the correct size of a reproductively active female, were released due to their overall poor body condition.

Reproductive characteristics can be used as evidence to support phylogenetic hypotheses (Palmer and Guillelte, 1992; Sever and Hamlett, 2002). Our data support the relationship between crocodilians and birds, as well as some similarities with turtles. The structure of the bird and alligator oviducts, with regard to shell formation, shows distinct variation from that of other reptiles. Further studies on crocodilian oviductal morphology are needed to make a proper comparison across the entire group and to determine if there are major differences between the more temperate and tropical species of crocodilians.

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