

Seasonal Variation in the Oviduct of Female *Agkistrodon piscivorus* (Reptilia:Squamata): An Ultrastructural Investigation

Dustin S. Siegel¹* and David M. Sever²

¹Department of Biology, Saint Louis University, St. Louis, Missouri 63103

²Department of Biological Sciences, Southeastern Louisiana University, Hammond, Louisiana 70402

ABSTRACT The annual oviductal cycle of the Cottonmouth, *Agkistrodon piscivorus*, is described using electron microscopy. This is only the second such study on a snake and the first on a viperid species. Specimens were collected in reproductive and nonreproductive condition throughout the year and five ultrastructurally unique regions were recognized: the anterior infundibulum, posterior infundibulum, glandular uterus, nonglandular uterus, and vagina. Except for the anterior infundibulum and vagina, which exhibit no seasonal variation in ultrastructure, the oviduct becomes highly secretory at the start of vitellogenesis. This includes the entire luminal border of the uterus, the tubular glands of the glandular uterus, and the luminal border and sperm storage tubules of the posterior infundibulum. The secretory materials produced in the oviduct vary among regions of the oviduct, and also can vary among time periods in the same region of the oviduct. Variation is especially evident in the sperm storage tubules. Secretory activity in the sperm storage tubules ceases after ovulation, but the tubular glands of the glandular uterus remain secretory until parturition, at which time secretory activity in the varying sections of the oviduct decreases dramatically. After parturition, the oviduct remains in a dormant state until the next reproductive season. The seasonal variation in oviductal morphology mirrors the temperate primitive reproductive cycle known for some pitvipers. Uterine glands of *A. piscivorus* are more similar in secretory activity to those of an oviparous lizard than a viviparous colubrid snake, suggesting variation in uterine gland morphology between snakes of different families. *J. Morphol.* 269:980–997, 2008. © 2008 Wiley-Liss, Inc.

KEY WORDS: reptilia; serpentes; *Agkistrodon piscivorus*; ultrastructure; oviduct

Given that viviparity has evolved numerous times in squamates (Blackburn, 1985; Shine, 1985), the reproductive morphology of squamates presents an interesting comparative model of convergent evolution. Three comprehensive reviews have appeared on the reproductive morphology and function of squamate oviducts (Blackburn, 1998; Girling, 2002; Thompson et al., 2006); however, ultrastructural studies on the oviducts of squamates are limited to a few studies on the snakes in the Colubridae (Hoffman and Wimsatt, 1972; Halpert et al.,

1982; Perkins and Palmer, 1996; Sever and Ryan, 1999; Sever et al., 2000) and Viperidae (Siegel and Sever, 2008), and on lizards in the Gekkonidae (Bou-Resli et al., 1981; Girling et al., 1997, 1998), Scincidae (Sever and Hopkins, 2004; Adams et al., 2004; Adams et al., 2007a, b), and Polychrotidae (Sever and Hamlett, 2002). Only one investigation outlines the entire seasonal variation of the entire oviduct in a squamate with ultrastructural analysis (Sever et al., 2000). In that study seasonal variation in the anterior infundibulum, posterior infundibulum (sperm storage area), uterus, and vagina was described in the colubrid snake *Seminatrix pygaea* (Sever et al., 2000).

Following the previous histological framework from Siegel and Sever (2006, 2008), five regions of the oviduct in the viviparous viperid, *Agkistrodon piscivorus*, were investigated with electron microscopy throughout the reproductive and nonreproductive seasons. This included the anterior infundibulum, posterior infundibulum (sperm storage area), glandular uterus, nonglandular uterus, and posterior vagina. This study marks the first study of its kind on a viperid species and extends our understanding of comparative reproductive morphology in squamates.

An investigation on reproductive morphology of female *Agkistrodon piscivorus* also poses an interesting opportunity to examine the biennial breeding cycle that is found in females of this species (Burkett, 1966; Wharton, 1966; Ford, 2002; Ford et al., 2004) and of other temperate viperids (Bull and Shine, 1979). Theoretically, this means that during every month of the year, female *A. piscivorus* should be found in one of two reproductive conditions (nonreproductive and reproductive), and because the reproductive cycles of temperate pitvipers have been reviewed extensively (Aldridge

*Correspondence to: Dustin S. Siegel, Department of Biology, Saint Louis University, St. Louis, MO 63103-2010. E-mail: dsiegel2@slu.edu

Published online 19 May 2008 in Wiley InterScience (www.interscience.wiley.com) DOI: 10.1002/jmor.10638

and Duvall, 2002), this study creates an interesting opportunity to correlate morphological variation with the reproductive and nonreproductive seasons.

Female viperids in temperate climates have been found to begin vitellogenesis in the late summer to early fall (for review see Aldridge and Duvall, 2002), which has been confirmed for female *Agkistrodon piscivorus* (Burkett, 1966; Wharton, 1966). Sperm also become present in the female oviduct at this time (Rahn, 1942; Gloyd and Conant, 1990; Siegel and Sever, 2006). After the fall mating season, hibernation ensues and further development of ovarian follicles ceases in temperate pitvipers (Aldridge and Duvall, 2002). Subsequent to hibernation, sperm can again become present in the oviduct signifying a spring breeding season, which has previously been observed in the oviduct of *A. piscivorus* females (Siegel and Sever, 2006, 2008). Ovarian follicles continue development until the time of ovulation in late spring (Aldridge and Duvall, 2002). Like other temperate pitvipers, female *A. piscivorus* are gravid until late summer/early fall when parturition occurs (Beyer, 1893; Conant, 1933; Allen and Swindell, 1948; Wharton, 1960; Funk, 1964; Burkett, 1966; Gloyd and Conant, 1990; Ford, 2002; Ford et al., 2004). By combining what is known about reproduction in female *A. piscivorus* with what is known about reproduction in temperate pitvipers in general, it is clear that the reproductive cycle of *A. piscivorus* mirrors that of the temperate zone primitive pattern outline by Aldridge and Duvall (2002).

MATERIALS AND METHODS

Specimens

Snakes were collected every month of the year (except hibernation months December and January) from three localities; the Amite River Diversion Canal (North 30°22.616/West 090°68.506, Livingston Parish, LA), Turtle Cove Environmental Research Station on Pass Manchac (North 30°29.426/West 090°35.592, Tangipahoa Parish, LA), and the private residence of Dr. Clifford Fontenot (10 km Northwest of New Albany, North 30°30.871/West 090°36.202, Livingston Parish, LA). Specimens were housed in glass aquaria (~0.3 m × 0.6 m × 0.3 m) with locking screen lids for no more than 3 days until euthanized. Water was available to these specimens ad libitum. Animals were euthanized by a 0.2–0.5 ml intraperitoneal injection of sodium pentobarbital (1 g sodium pentobarbital in 10% alcohol, 40% propylene glycol solution).

The right side of the oviduct was removed and fixed 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 examination. The number of sexually mature specimens prepared per month was: February, 1; March, 2; April, 3; May, 2; June, 2; July, 2; August, 3; September, 1; October, 2; November, 2. Snout-vent lengths (SVL) ranged from 47.3 to 67.8 cm.

Microscopy

Histochemistry. Methods for outlining the seasonal variation in histochemistry of the oviduct in *Agkistrodon piscivorus*

can be found in Siegel and Sever (2008). A periodic acid Schiff (PAS) procedure was used to identify neutral carbohydrates, Alcian blue 8GX (AB) was used at pH 2.5 to identify carboxylated glycosaminoglycans, and bromophenol blue (BB) to demonstrate proteins.

Ultrastructure. Tissues fixed in Trump's solution were rinsed in deionized water and then postfixed for 90 min in 2% osmium tetroxide. They were then rinsed in deionized water, dehydrated with a graded series of ethanol (70, 95, 100% for 1 h each), soaked 30 min each in 1:1 of 100% ethanol:propylene oxide, followed by pure propylene oxide, and subsequently embedded in Epon (EmBed 812, Electron Microscopy Sciences, Fort Washington, PA) for ultra-thin sectioning with a RMC MT7 ultramicrotome (Research and Manufacturing, Tucson, AZ). Tissues were critically point dried immediately after dehydration with a DCP-1 critical point drier (Denton Vacuum, Moorestown, NJ) for scanning electron microscopy. Ultra-thin sections were taken from the Epon embedded tissue at 70 nm with a DiATOME diamond knife (DiATOME, Biel, Switzerland), placed on copper grids, and stained with uranyl acetate and lead citrate for examination under a JEOL JEM 100s transmission electron microscope (JEOL, Peabody, MA). Critically point dried tissues were mounted on stubs, sputter coated with a Denton Desk IV XLS (Denton Vacuum, Moorestown, NJ), and viewed with a Philips XL-20 scanning electron microscope (Philips Electronics N.V., Eindhoven, Netherlands).

Kodak Film (Eastman Kodak, Rochester, NY) was used for TEM photography and was developed in Kodak developer for 4 min, rinsed in water for 30 s, fixed in Kodak fixer for 4 min, and then rinsed in water for 20 min. Negatives were then allowed to dry and then scanned directly into Adobe Photoshop version 7.0 (Adobe Systems, San Jose, CA) for editing and printing. Photographs for SEM photography were obtained digitally and downloaded directly into Adobe Photoshop version 7.0.

RESULTS

Reproductive Cycle

Agkistrodon piscivorus females follow a temperate zone primitive pattern with late summer/fall and spring mating periods prior to ovulation in late spring. Vitellogenesis begins in these snakes in late summer/early fall with follicular growth continuing until hibernation. At this time, follicles arrest in development and continue growth subsequent to hibernation until a max size is reached at the time of ovulation. Gestation and embryogenesis occur until the late summer/fall where parturition takes place (see Fig. 1 for review of vitellogenic and embryogenic cycle). A review of the histochemical analysis compiled from earlier work (Siegel and Sever, 2008) shows that the late summer/fall mating season is correlated with the start of secretory activity in multicellular glands in the oviduct. This includes PAS+ secretory activity in the sperm storage tubules of the infundibulum, and PAS+/BB+ activity in the uterine glands of the glandular uterus. There was no variation throughout the reproductive and nonreproductive seasons in histochemical analysis from epithelium of the nonglandular uterus (AB+) and vagina (PAS+/AB+). The infundibular glands and epithelium lining the lumen varied in PAS/AB secretory activity; however, this activity did not mirror the reproductive cycle. The following sections outline the ultrastructure in the five regions of the oviduct

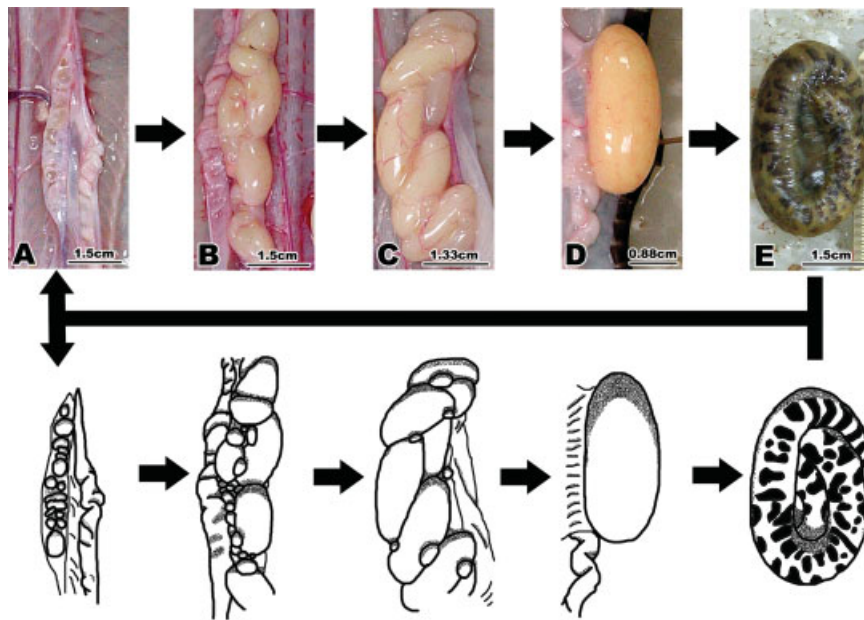


Fig. 1. Follicle development and embryogenic cycle in female *Agkistrodon piscivorus*. (A) Ovarian follicle size in postpartum to late summer/fall mating season females (example: August 2005–September 2006). (B) Ovarian follicle size immediately prior to hibernation (example: November 2006). (C) Ovarian follicle size shortly after emergence from hibernation (example: March 2007). (D) Ovarian follicle size immediately prior to ovulation (example: May 2007). (E) Near fully developed embryo removed from uterus in August (gravidity would occur from May to August/September).

during the reproductive and nonreproductive seasons of female *Agkistrodon piscivorus*.

Ultrastructure

Anterior infundibulum. The anterior infundibulum exhibits no seasonal variation in ultrastructure. The entire infundibulum is characterized by a very thin muscularis externa and mucosa with a simple squamous epithelium. The epithelium of infundibular glands formed in this anterior region is ultrastructurally identical to the epithelium of the lumen. Throughout the year, the epithelium contains abundant lipid material (Fig. 2A,B). This lipid material is either highly organized into electron-dense ovoid droplets or unorganized electron lucent material (Fig. 2B). Epithelial secretory cells with microvilli, and ciliated cells are found to be interspersed (Fig. 2A). Smooth endoplasmic reticulum is abundant around the nuclear region of the secretory cells (Fig. 2C). Ciliated cells contain numerous small mitochondria associated with basal bodies, which anchor the cilia (Fig. 2D). Nuclei of all the epithelium cells are euchromatic and are located basally (Fig. 2A,B). Epithelial cells are connected apically by tight junctions and desmosomes, and intercellular canaliculi (Fig. 2D) are narrow and convoluted.

Posterior infundibulum (sperm storage area). The cuboidal epithelial cells of the tubular glands in the posterior infundibulum (sperm storage tubules) become highly secretory during the mating seasons of *Agkistrodon piscivorus*. Secretory vacuoles appear nonelectron dense at the beginning of the fall mating season and turn electron dense shortly thereafter (Fig. 3A,B). At this time Golgi complexes become highly visible apical to

the basally located heterochromatic nuclei of the secretory cells (Fig. 3C). Small mitochondria can also be observed in the cytoplasm (Fig. 3D). At the start of vitellogenesis, lipid droplets can be found in a basally located position in the secretory cells (Fig. 3E). The secretory cells of the epithelium lining the lumen exhibit this same alteration except no lipid material was ever observed. Ciliated cells show no seasonal variation in ultrastructure, are found alternating with secretory cells, and are ultrastructurally identical to those found in the anterior infundibulum. These cells are greatest in concentration at the opening of the sperm storage tubules. Tight junctions and desmosomes can be observed adhering these secretory and ciliated cells together in the epithelium lining the lumen and in the sperm storage tubules. Intercellular canaliculi are narrow in this region like those of the anterior infundibulum.

Shortly after the beginning of copulation, sperm can be found in the sperm storage tubules of the posterior infundibulum (Fig. 3A,B). As sperm move deeper into these tubular glands, they become aligned with their nuclei facing the most distal portion of the sperm storage tubules, which is composed primarily of secretory cells. A secretory matrix can be seen associated with sperm throughout the time of its presence in these sperm storage tubules. After ovulation, secretory activity in the sperm storage tubules ceases. However, sperm can remain present until the next reproductive season. See Siegel and Sever (2008) for a review on sperm storage in female *Agkistrodon piscivorus*.

Glandular uterus. The glandular uterus is characterized by a simple cuboidal epithelium, a rather thick lamina propria and muscularis

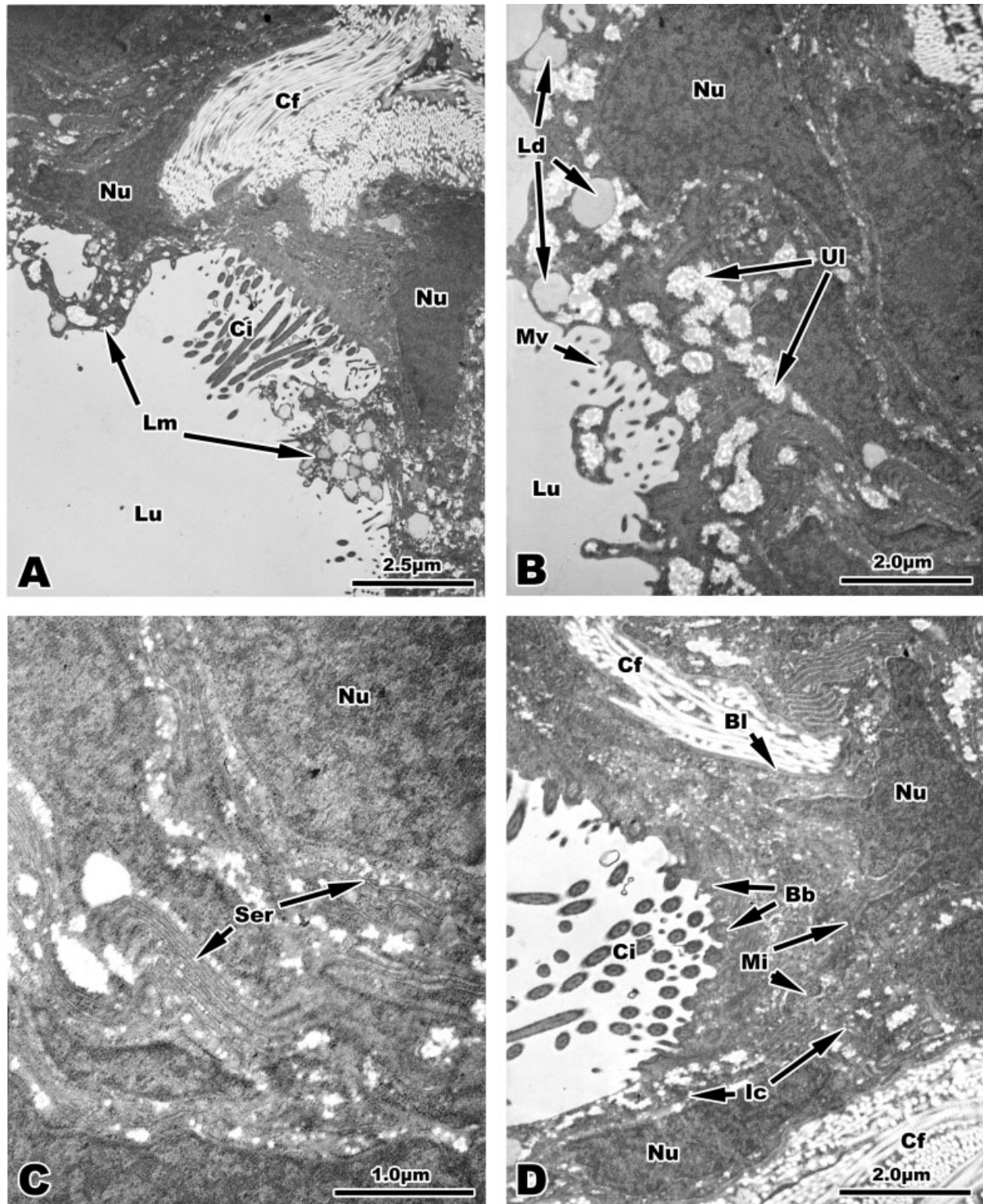


Fig. 2. Ultrastructure of the anterior infundibulum in female *Agkistrodon piscivorus*. (A) Overview of the anterior infundibulum in an October female. (B) Higher magnification of (A) demonstrating two types of lipid material. (C) Higher magnification of (B) emphasizing abundant smooth endoplasmic reticulum in the cytoplasm of secretory cells. (D) Higher magnification of (A) showing ultrastructure of ciliated cells. Bb, basal bodies; Bl, basal lamina; Cf, collagen fiber; Ci, cilia; Ic, intercellular canaliculi; Ld, lipid droplet; Lm, lipid material; Lu, lumen; Mi, mitochondria; Mv, microvilli; Nu, nucleus; Ser, smooth endoplasmic reticulum; UI, unorganized lipid material.

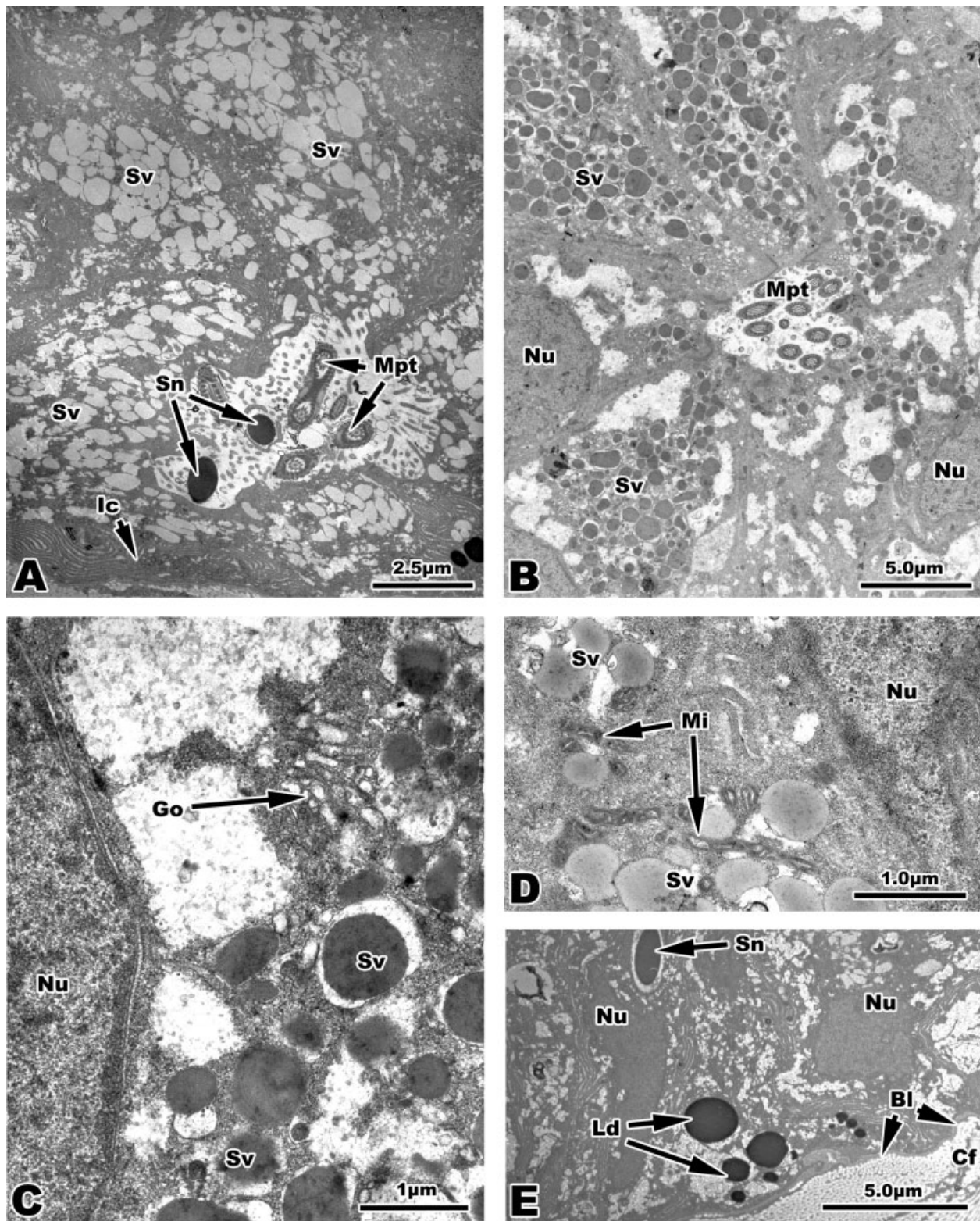


Fig. 3. Ultrastructure of sperm storage tubules in the posterior infundibulum of female *Agkistrodon piscivorus*. (A) Overview of a sperm storage tubule in an October female. (B) Overview of a sperm storage tubule in a May female. (C,D) Higher magnification of (B) showing the cytoplasmic contents of secretory cells in a sperm storage tubule. (E) Basal portion of the epithelium in an October female emphasizing lipid droplets in the cytoplasm. Bb, basal bodies; Bl, basal lamina; Ci, cilia; Go, Golgi complex; Ic, intercellular canaliculi; Ld, lipid droplets; Mi, mitochondria; Mpt, middle piece of the tail; Sn, sperm nuclei; Sv, secretory vacuoles.

externa compared to that of the infundibulum, and abundant simple tubular glands. The epithelium lining the lumen is composed primarily of secretory cells with ciliated cells randomly dispersed (Fig. 4A). Scant amount of sperm can be found in this area during the mating season (Fig. 4A).

Shortly after the start of mating season, the tubular glands of the glandular uterus become hypertrophied and contain mildly electron-dense secretory vacuoles (Fig. 4B,C) whose products are secreted in a merocrine manner. During this time of the year abundant rough endoplasmic reticulum can be observed surrounding the basally positioned, euchromatic nuclei of the tubular gland cells (Fig. 4E). The nuclei all contain a centrally located nucleolus (Fig. 4C), and numerous small mitochondria are dispersed in the cytoplasm (Fig. 4E). Uterine gland hypertrophy commences at the summer/fall mating season, continues on through the spring mating season, and potentially throughout gravidity. Unfortunately, reproductively active females were not obtained from every month of gravidity. Therefore, uterine gland activity throughout gravidity is inferred by the presence of hypertrophied uterine glands containing a protein-positive secretory material (like the material from other months of reproductive activity) in a late August gravid female (courtesy of the Louisiana Museum of Natural History).

The high level of secretory activity that occurs in the tubular glands is mirrored by the secretory cells of the epithelium lining the lumen of the glandular uterus (Fig. 5A–C). However, the secretions in these secretory cells are more electron lucent than those of the tubular glands (Fig. 5A). Microvilli cover the luminal boarder of the secretory cells and the mode of secretion of the epithelium is apocrine (Fig. 5A–C). Cells without secretory vacuoles contain oversized mitochondria and numerous Golgi bodies (Fig. 5C), indicative of a high rate of secretory material production and release. Tight junctions and desmosomes connect the epithelial cells apically (Fig. 5C), and narrow to large intercellular canaliculi exist between the cells of the epithelium (Fig. 5B,C). Ciliated cells are dispersed throughout the luminal epithelium and contain numerous mitochondria associated with basal bodies anchoring the cilia. Nuclei of these cells are euchromatic and basally located like those of the secretory cells of this region. Sperm present in the uterus are almost always associated with a dense secretory matrix of unknown origin (Fig. 5D).

After parturition in the late summer/fall, secretory activity in the glandular uterus decreases dramatically. The secretory cells of the epithelium lining the lumen contain a decreased amount of secretory vacuoles (Fig. 6A), while the cilia remain ultrastructurally identical to those of the reproductive season (Fig. 6B). The secretory cells of the tu-

bular glands contain no secretory vacuoles (Fig. 6C,D), and lipofuscin can be observed in the cytoplasm of these cells (Fig. 6D) and in those of the luminal epithelium (Fig. 6A,D). The glandular uterus remains in this state until the next fall when the next reproductive season begins.

Nonglandular uterus. The nonglandular uterus is characterized by a thick muscularis externa that is much greater in width compared to that of the glandular uterus. This region is composed entirely of a simple epithelium and the epithelial cells alter from cuboidal to columnar when moving anterior to posterior in the oviduct. No tubular invaginations forming glands are present in the nonglandular uterus. The epithelium of the nonglandular uterus, which is composed entirely of alternating ciliated and secretory cells (Fig. 7A), varies in three distinct stages throughout the year. In late summer, at the start of the mating season, alternating ciliated and secretory epithelial cells contain lipofuscin and can be observed desquamating from the basal lamina (Fig. 7B,C). Large circular mitochondria can be observed in both desquamating secretory and ciliated cell types and basal bodies of the cilia can be seen (Fig. 7C); however, no longer are cilia attached to these basal bodies (Fig. 7C). Secretory cells during this time of the year contain little to no secretory material (Fig. 7B).

The next stage of the nonglandular uterus cycle is the production of mildly electron-dense secretory vacuoles in the fall. These vacuoles dominate the apical portion of the cells while nuclei are present basally (Fig. 7D). Golgi complexes are visible during this time period as are small mitochondria. Ciliated cells are ultrastructurally identical to those found in all other regions of the oviduct at this stage (Fig. 7D).

After hibernation, secretory activity in the nonglandular uterus increases with apocrine blebs being secreted into the lumen (Fig. 7E). Organelles responsible for secretion synthesis are abundant in cells that have no secretory material, which indicates the rapid production and release of secretory product at this time. Conspicuous organelles include large irregularly-shaped mitochondria and high concentrations of Golgi complexes.

Like the glandular uterus, sperm can be found in this region during times of mating activity (Fig. 8A). The abundance of sperm is much greater than that of the glandular uterus; however, sperm in this area undergo degradation before the time of ovulation. For review of these sperm aggregations in female *Agkistrodon piscivorus*, see Siegel and Sever (2008).

Mast cells are numerous in the lamina propria of the nonglandular uterus at all times of the year and contain numerous osmiophilic, polyhedral, secretory vacuoles with a peroxidase-composed core. The secretory vacuoles typically obscure cytoplas-

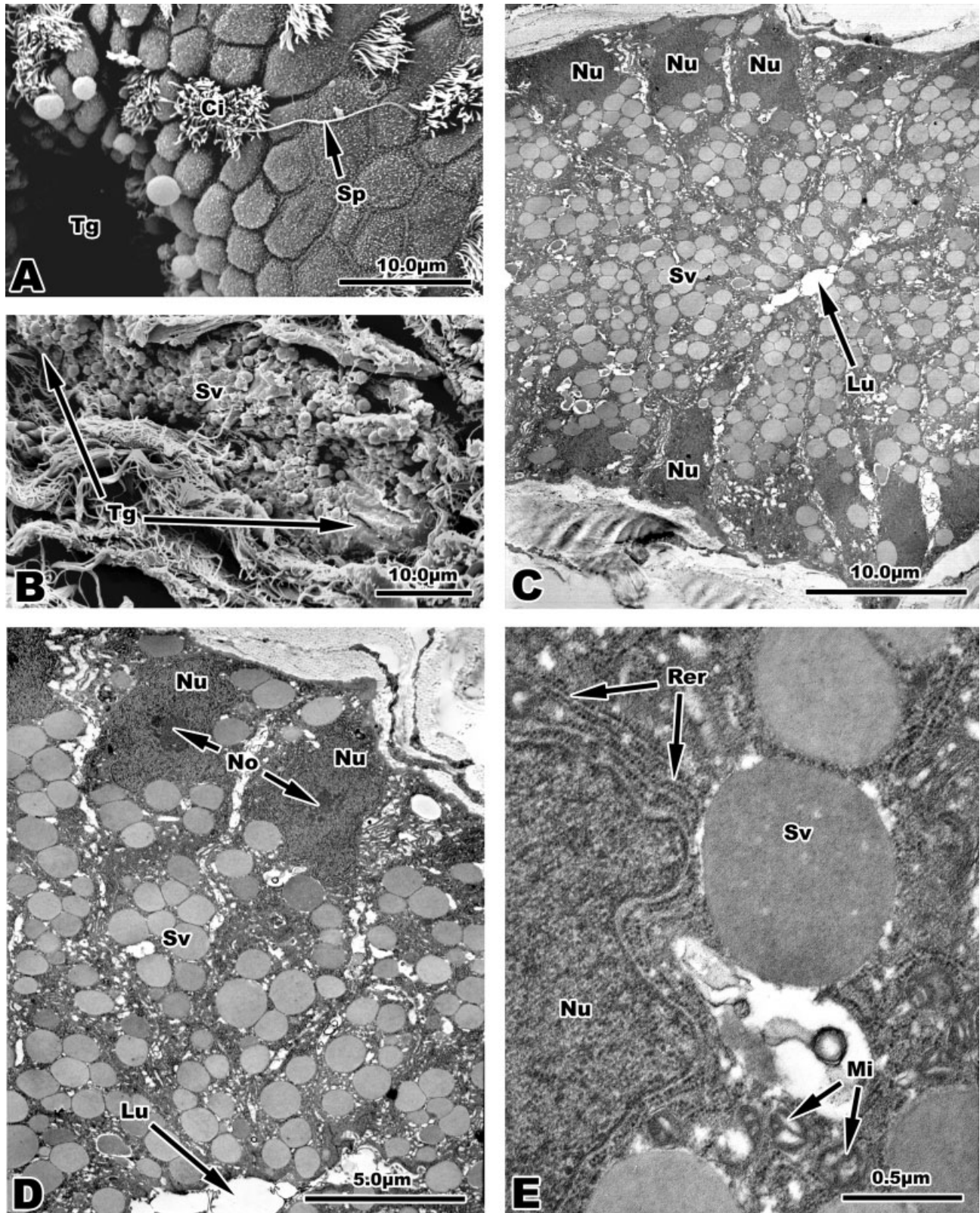


Fig. 4. Ultrastructure of the tubular glands in the glandular uterus of female *Agkistrodon piscivorus*. (A) Overview of the surface of the uterus in an October female. (B) Overview of a tubular gland in the uterus of a May female. (C) Overview of a tubular gland in an April female. (D) Higher magnification of (C). (E) Higher magnification of (C) demonstrating the cytoplasmic contents of the secretory cells in the tubular glands. Ci, cilia; Lu, lumen; Mi, mitochondria; No, nucleolus; Nu, nucleus; Rer, rough endoplasmic reticulum; Sp, sperm; Sv, secretory vacuoles; Tg, tubular gland.

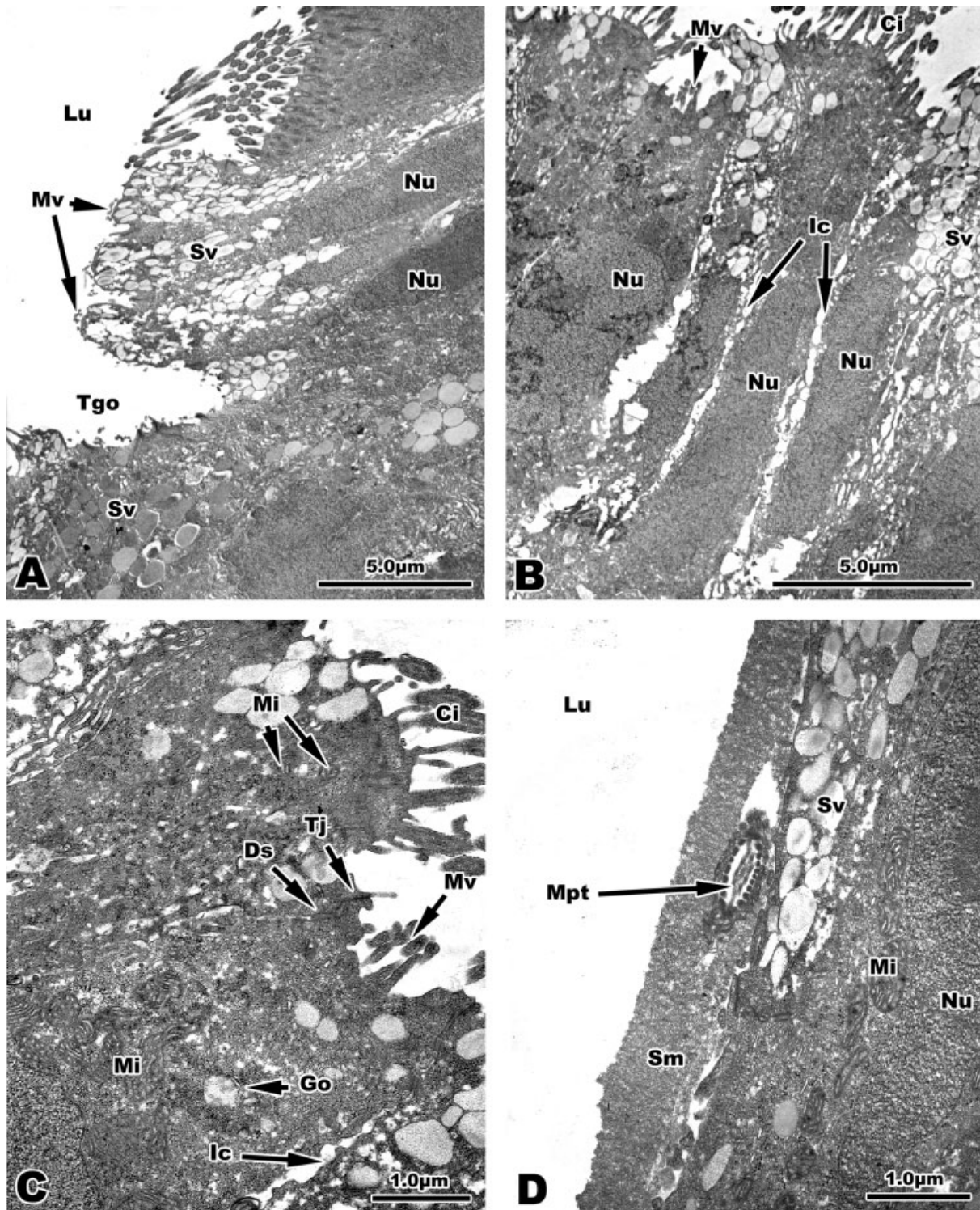


Fig. 5. Ultrastructure of the luminal boarder of the glandular uterus during times of high secretory activity in female *Agkistrodon piscivorus* (all from the month of April). (A) Overview of the electron-lucent secretory vacuoles compared to the electron-dense secretory vacuoles observed at the opening of a tubular gland. (B) Overview of the luminal boarder. (C) Higher magnification of (B) emphasizing the cytoplasmic contents of the secretory cells. (D) The presence of sperm surrounded by a secretory material in the lumen of a reproductively active female. Ci, cilia; Ds, desmosome; Go, Golgi complex; Ic, intercellular canaliculi; Lu, lumen; Mi, mitochondria; Mpt, middle piece of tail; Mv, microvilli; Nu, nucleus; Sv, secretory vacuoles; Tgo, tubular gland opening; Tj, tight junction.

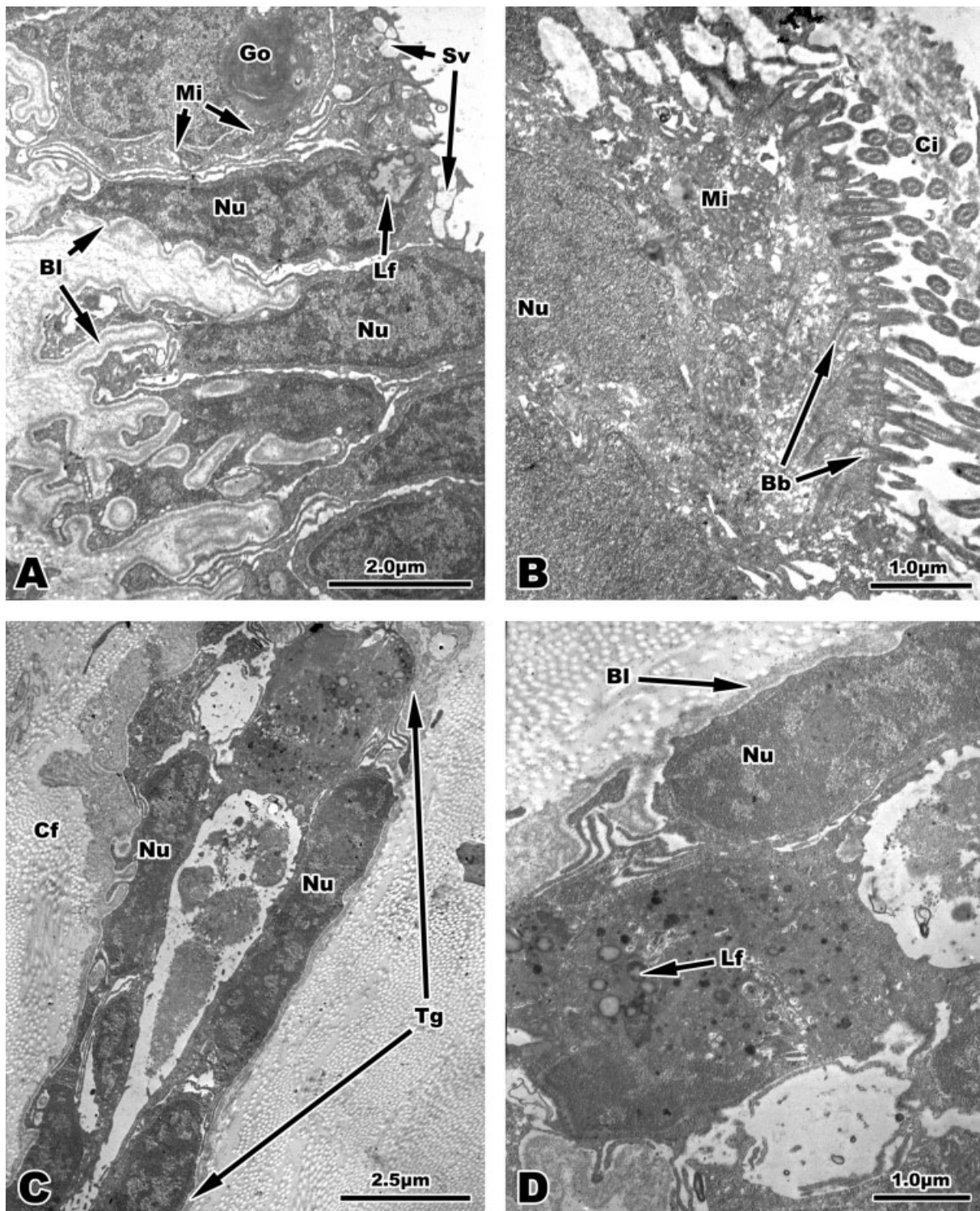


Fig. 6. Decreased activity before the onset of the reproductive cycle and the ultrastructure of ciliated cells in the glandular uterus in female *Agkistrodon piscivorus*. (A) Overview of secretory cells of the luminal epithelium demonstrating the presence of scant secretory vacuoles in an August female. (B) High magnification of ciliated cells on the luminal border of the epithelium in a March female. (C) Overview of a tubular gland in an August female showing the decreased secretory activity. (D) Higher magnification of (C) exhibiting the cytoplasmic content in secretory cells of a tubular gland. Bb, basal bodies; Bl, basal lamina; Cf, collagen fiber; Ci, cilia; Go, Golgi complex; Lf, lipofuscin; Mi, mitochondria; Nu, nucleus; Sv, secretory vacuoles; Tg, tubular gland.

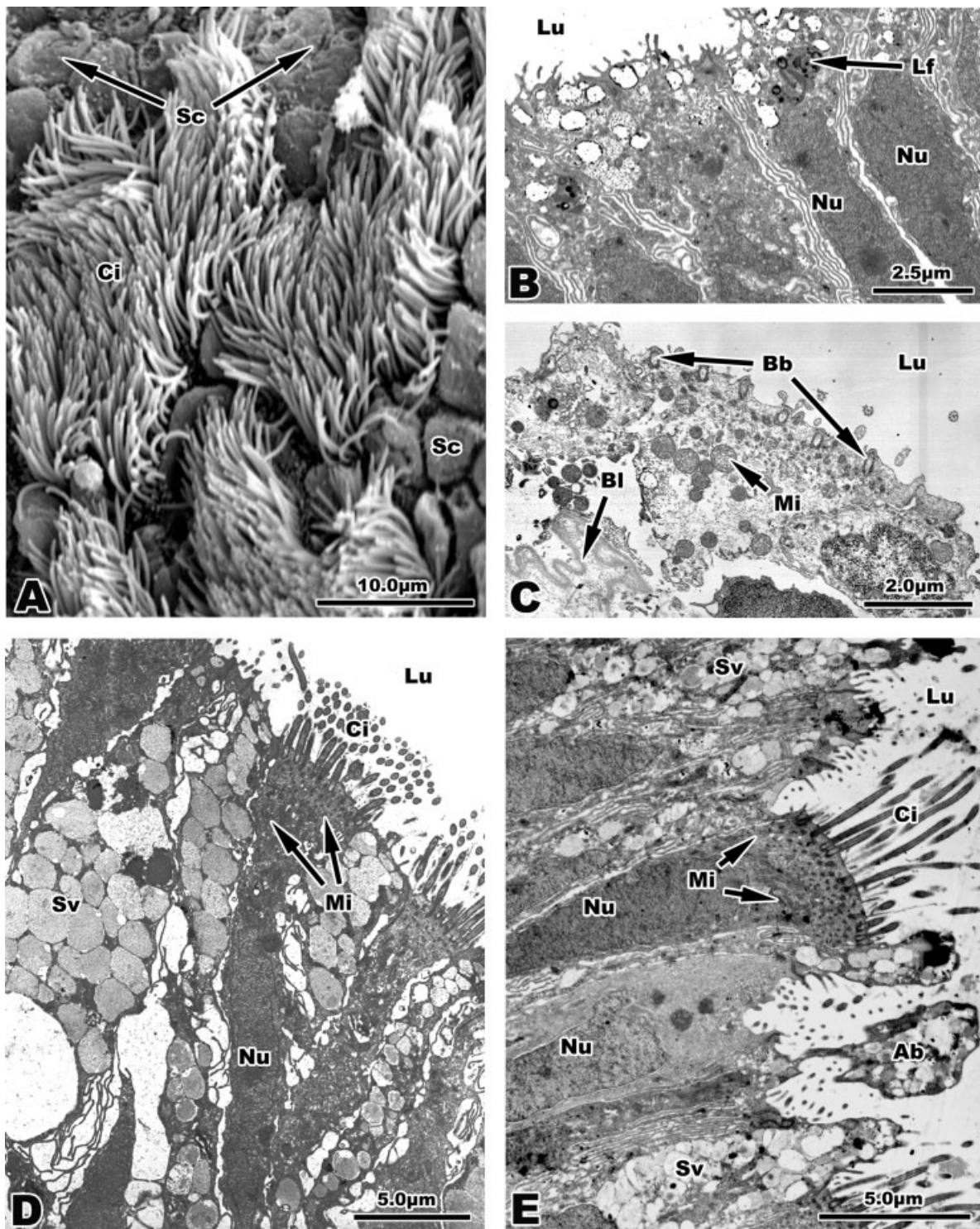


Fig. 7. Seasonal variation in the ultrastructure of the non-glandular uterus in female *Agkistrodon piscivorus*. (A) Overview of the surface of the epithelium in an October female. (B) Overview of the epithelium of secretory cells showing scant secretory material and lipofuscin in the cytoplasm in a July specimen. (C) Desquamation of a ciliated cell and its cytoplasmic contents in an August female. (D) Overview of the epithelium of an October female demonstrating an increased amount of secretory material. (E) Increased activity of the secretory cells in a May specimen exhibiting blebbing of secretory vacuoles and cytoplasm into the lumen. Ab, apocrine bleb; Bb, basal bodies; Bl, basal lamina; Ci, cilia; Lf, lipofuscin; Lu, lumen; Mi, mitochondria; Nu, nucleus; Sc, secretory cell; Sv, secretory vacuoles.

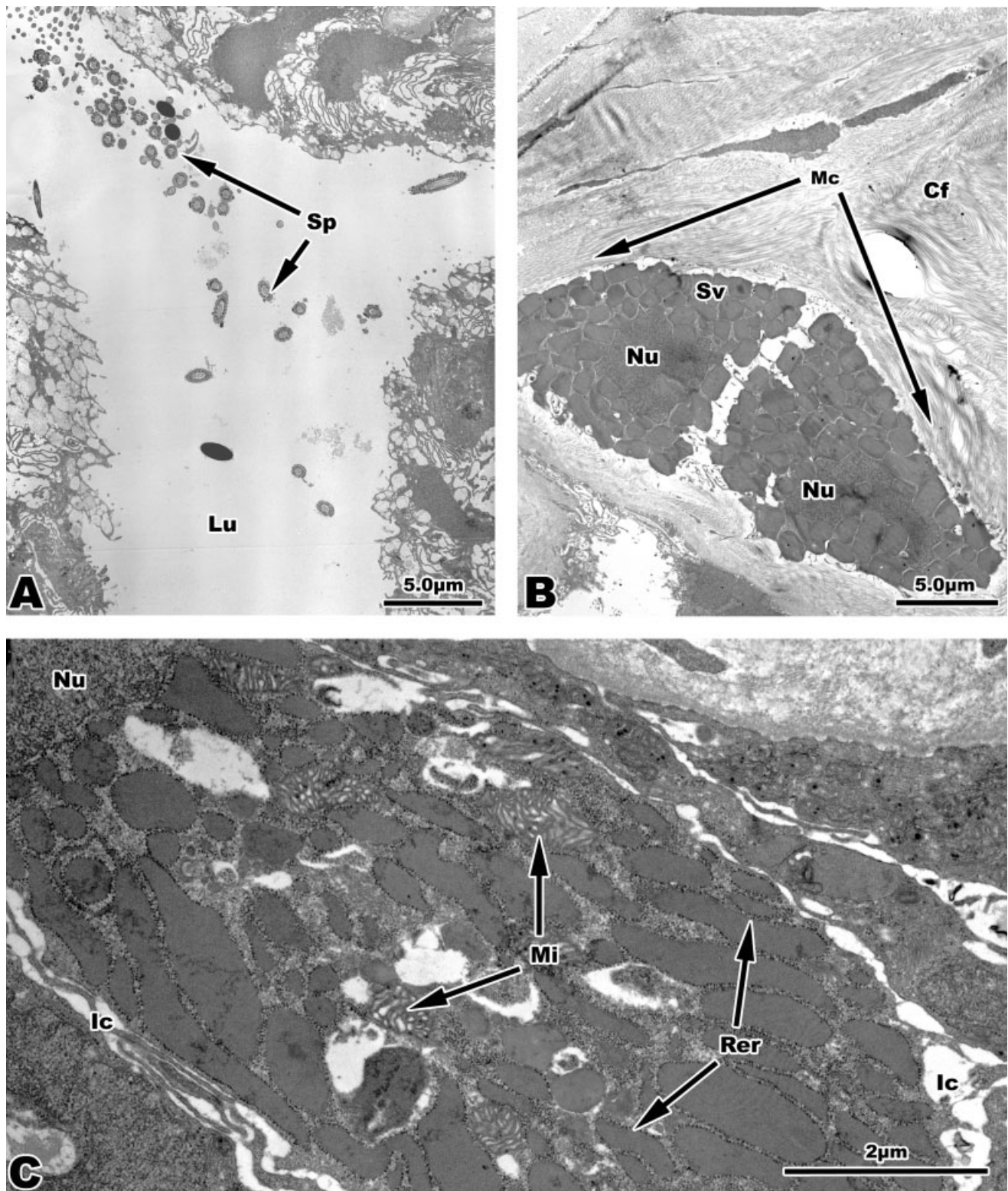


Fig. 8. The presence of sperm and anti-inflammatory cells in the non-glandular uterus of female *Agkistrodon piscivorus*. (A) Sperm in the lumen of a July specimen. (B) Mast cells in the lamina propria of a July specimen. (C) Plasma-like cells invading the complex intercellular canaliculi in a May specimen. Cf, collagen fiber; Cr, cisternae of rough endoplasmic reticulum; Ic, intercellular canaliculi; Lu, lumen; Mc, mast cells; Mi, mitochondria; Nu, nucleus; Rer, rough endoplasmic reticulum; Sp, sperm; Sv, secretory vacuoles.

mic contents, although small mitochondria, scattered cisternae of rough endoplasmic reticulum, and euchromatic nuclei can occasionally be observed (Fig. 8B; see Fig. 10D for cytoplasmic contents). Plasma cells filled with rough endoplasmic reticulum and large mitochondria invade the basal portion of the epithelium throughout the year and reside in the complex intercellular canaliculi between epithelial cells of the nonglandular uterus (Fig. 8C).

Vagina. The vagina is the most posterior portion of the oviduct and is characterized by a highly secretory epithelium composed of columnar cells. This region contains a very thick lamina propria and muscularis externa. The vaginal epithelial cells exhibit no seasonal variation in secretory activity and abundant secretory vacuoles fill these cells at all times of the year (Fig. 9B,C). The secretory vacuoles are mildly electron dense and often contain a highly electron-dense core (Fig. 9C). The secretory material is released by merocrine secretion and then lines the microvilli-covered luminal border of the epithelium (Figs. 9C and 10C). A few ciliated cells can be found intermixed with the secretory cells and are ultrastructurally identical to those in other portions of the oviduct (Fig. 10A). In the secretory epithelial cells euchromatic nuclei are located basally and are typically surrounded by lipid droplets (Fig. 10B). The epithelial cells are united by tight junctions and desmosomes apically (Fig. 10C). Mast cells identical to those found in the nonglandular uterus (Fig. 10D) are highly concentrated in the lamina propria of the vagina. At the most basal portion of the epithelium, adjacent to the basal lamina, the intercellular canaliculi increase in size and plasma-like cells, identical to the ones found in the nonglandular uterus invade this area (Fig. 10B). Sperm are present in the most anterior portion of the vagina during the mating seasons.

DISCUSSION

The Female Reproductive Cycle

Ultrastructural and histochemical analysis of the oviduct, combined with visual evidence of vitellogenesis, confirms the hypothesis that female *Agkistrodon piscivorus* operate under the temperate zone primitive pattern described by Aldridge and Duvall (2002). The reproductive season begins in late summer to early fall with copulation, and shortly after, hypertrophy and increased secretory activity occur in the uterus and sperm storage tubules of the posterior infundibulum. Sperm migrate to specialized sperm storage tubules in the posterior infundibulum and remain in these glands through hibernation, a phenomenon known as long-term sperm storage (Halpert et al., 1982).

In the spring, female *Agkistrodon piscivorus* emerge from hibernation and enter a second mating season, which is indicated by the reoccurrence

of sperm in the posterior oviduct. These sperm would therefore qualify as short-term storage sperm (Halpert et al., 1982). Therefore, long-term sperm storage sperm mixing with short-term sperm storage sperm is a possibility. Not only are long-term sperm storage and short-term sperm storage important strategies utilized by different snake species, these strategies are also important within the same species (Halpert et al., 1982) and possibly same individual. However, it is not known if any one female *A. piscivorus* will mate in the fall and spring or in just one season, or if this situation varies among individuals and populations. Whether sperm from long-term sperm storage or short-term sperm storage have a higher fertilization success is also unknown. However, multiple paternity has been shown to occur in Colubridae (Gibson and Falls, 1975; Barry et al., 1992) and Viperidae (Stille et al., 1986), and it has been hypothesized that a first male advantage is present in *Vipera berus* (Stille et al., 1986; Stille and Niklasson, 1987). The latter studies did not determine whether first male advantage involved long-term sperm storage or short-term sperm storage because artificial mating conditions were used. Halpert et al. (1982) reported that sperm from autumn mating in *Thamnophis sirtalis* were evacuated from infundibular sperm storage tubules and replaced with sperm from spring mating. Whether an evacuation of long-term sperm storage sperm occurs in *A. piscivorus* could not be determined definitively in this study, but we consider the possibility unlikely, as sperm are continuously present in sperm storage tubules of reproducing females from the onset of mating through parturition.

The oviduct remains at a high level of secretory activity until ovulation at the end of spring, at that point secretory activity decreases in the lumen of the glandular and nonglandular uterus and ceases altogether in the sperm storage tubules of the posterior infundibulum. However, based on earlier light microscopy work (Siegel and Sever, 2008), the tubular glands of the uterus secrete a protein substance throughout gravidity. Lipid secretions in the anterior infundibulum and mucoid secretions in the vagina do not vary with the reproductive cycle.

After parturition in the fall (Beyer, 1893; Conant, 1933; Allen and Swindell, 1948; Wharton, 1960; Funk, 1964; Burkett, 1966; Gloyd and Conant, 1990; Ford, 2002; Ford et al., 2004), the tubular glands finish secretory activity and the oviduct remains inactive until the next late summer/fall mating season. Sperm remain in the sperm storage tubules until the start of the next mating season of this biennially-breeding species, at that point they disappear. Therefore, sperm have the potential of remaining in sperm storage tubules for as long as 22 months (example: August 2005–June 2007). However, because sperm storage tubule secretory activity ceases subsequent to ovulation,

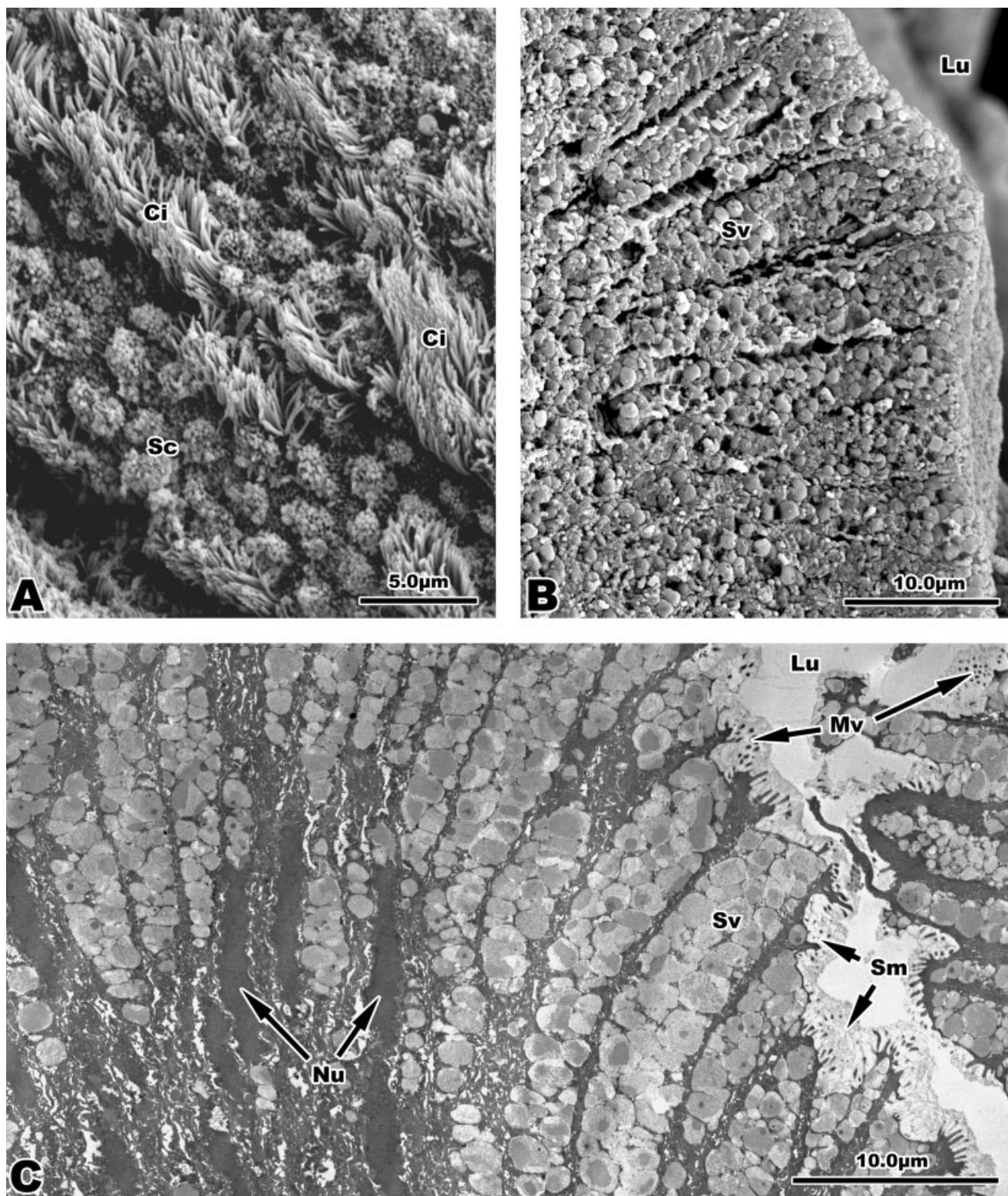


Fig. 9. Ultrastructure of the vaginal region in female *Agkistrodon piscivorus*. (A) Overview of the epithelial surface in an October female. (B) Cross-section of the vaginal epithelial cells exhibiting abundant secretory vacuoles in a May specimen. (C) Overview of the secretory epithelial cells in the vagina showing abundant secretory vacuoles and secretory material lining the surface of the epithelium in a March specimen. Ci, cilia; Lu, lumen; Mv, microvilli; Nu, nucleus; Sc, secretory cell; Sm, secretory material; Sv, secretory vacuoles.

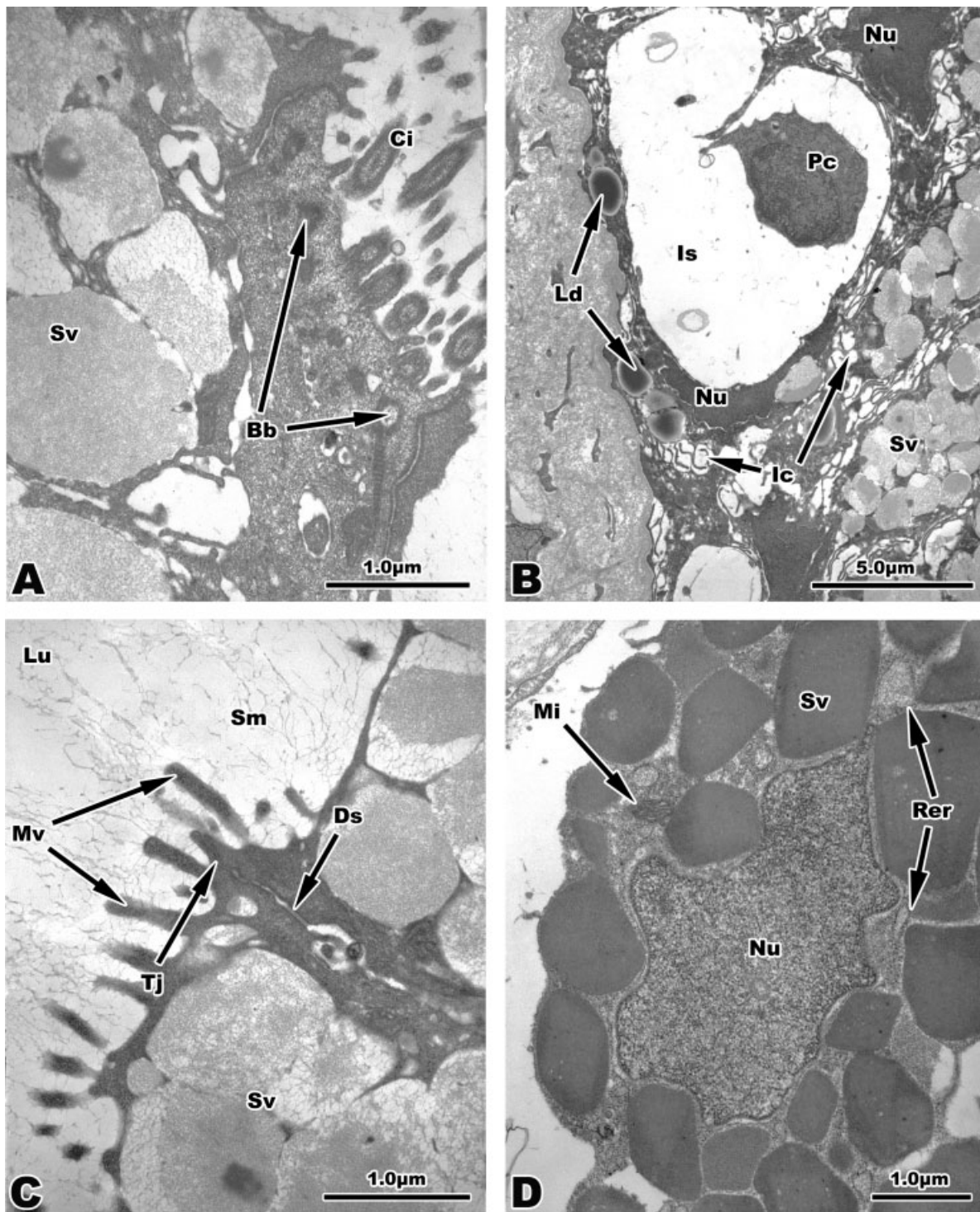


Fig. 10. Ultrastructural characteristics of the vagina in female *Agkistrodon piscivorus*. (A) High magnification of a ciliated cells interspersed between two secretory cells in an April specimen. (B) The invasion of a plasma-like cell into intercellular space and basally located lipid droplets in an April specimen. (C) High magnification of the apex of the vaginal epithelium exhibiting secretory material lining the surface of the epithelium and the junctions between the epithelial cells in an April female. (D) A mast cell in the lamina propria in an April specimen. Bb, basal bodies; Ci, cilia; Ds, desmosome; Ic, intercellular canaliculi; Is, intercellular space; Ld, lipid droplets; Lu, lumen; Mi, mitochondria; Mv, microvilli; Nu, nucleus; Pc, plasma-like cell; Rer, rough endoplasmic reticulum; Sm, secretory material; Sv, secretory vacuoles; Tj, tight junction.

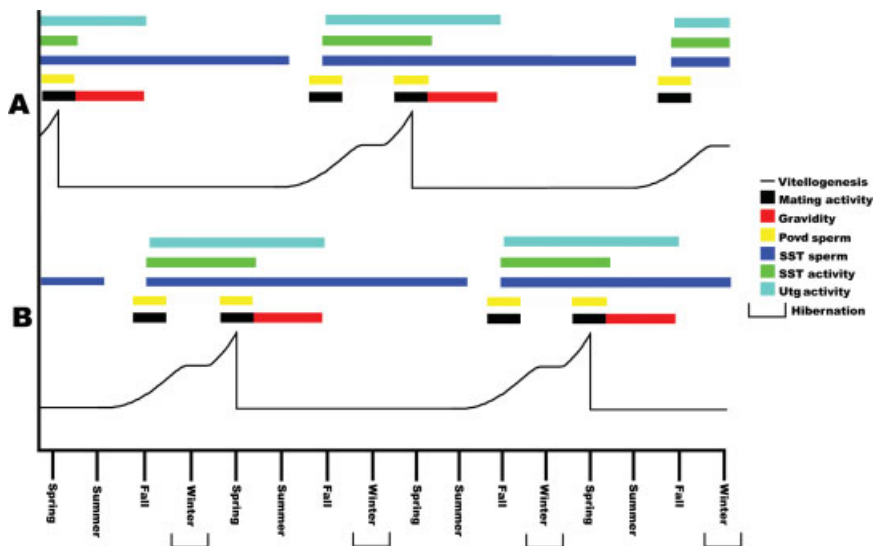


Fig. 11. Reproductive cycle of female *Agkistrodon piscivorus* (redrawn from Aldridge and Duvall, 2002). Biennial cycle of reproduction in *A. piscivorus* females results in two possible reproductive conditions in each month (A or B). Povid sperm, sperm presence in the posterior oviduct; SST sperm, sperm presence in SSTs; SST activity, secretory activity of sperm storage tubules; Utg activity, uterine gland secretory activity.

and the secretions are hypothesized to benefit sperm viability in snakes (Hoffman and Wimsatt, 1972) and in lizards (Cuellar, 1966), it is unlikely that these sperm remain viable for this extended period. Thus, sperm retention in female *Agkistrodon piscivorus* should not be considered as an example of very long-term sperm storage as proposed by Birkhead et al. (1993). The disappearance of sperm must either occur by spermiphagy by the sperm storage tubule epithelium or by natural degradation of sperm. However, neither of these mechanisms was observed in the sperm storage tubules in this study.

Because female *Agkistrodon piscivorus* are biennial breeders (Burkett, 1966; Wharton, 1966; Ford, 2002; Ford et al., 2004), females can be in one of two possible reproductive conditions during any month of the year: reproductive or nonreproductive. By redrawing Figure 1B from Aldridge and Duval (2002) and adding the presence of sperm in different regions of the oviduct and the secretory activity of the oviduct, the potential reproductive condition of any female can be determined (Fig. 11; keep in mind that these data represent extremes, not all snakes mate as early as July or retain sperm until the June of the next year). One quandary of our study is that the possibility of some female *A. piscivorus* reproducing annually or triennially cannot be ruled out; however, because of findings from previous investigations (Burkett, 1966; Wharton, 1966; Ford, 2002; Ford et al., 2004), we believe that the majority of female *A. piscivorus* are in fact biennial breeders.

How the Male Reproductive Cycle Coincides

In male *Agkistrodon piscivorus* studied in Alabama and Georgia, spermatogenesis occurs in the late summer/fall associated with a peak in testosterone levels (Johnson et al., 1982; Graham, 2006).

This coincides with the late summer/fall mating season when sperm appear in the reproductive tract of the females (Siegel and Sever, 2006, 2008), and an increase in secretory activity is observed in the uterine regions and sperm storage tubules of the posterior infundibulum. Considering that *A. piscivorus* in our population mate in the summer/fall and spring (evidenced by sperm in the posterior oviduct; Siegel and Sever, 2006), this would indicate that males must be capable of storing sperm in the ductus deferens over winter for the spring mating. Histological evidence of male *A. piscivorus* (Johnson et al., 1982; Graham, 2006) and other temperate male viperids (Diller and Wallace, 1984; Aldridge, 1993, 2002) shows sperm presence in the ductus deferens throughout the year. The timing of spermatogenesis between different taxa appears conserved in temperate viperids (Aldridge, 1979, 1993, 2002; Johnson et al., 1982; Aldridge and Brown, 1995), and this is probably due to the ability to store sperm in the ductus deferens until females become receptive.

Hypertrophy of the renal sexual segment in males coincides with the late summer/fall and spring mating seasons of *Agkistrodon piscivorus* in populations from Alabama (Johnson et al., 1982) and Louisiana (Sever et al., 2007). This pattern is similar in other investigations on viperid reproductive cycles (Aldridge, 2002). Interestingly, no statistical variation in renal sexual segment hypertrophy was observed in male *A. piscivorus* in Georgia, although a nonsignificant peak in renal sexual segment hypertrophy was noted in the late summer/fall during spermatogenesis (Graham, 2006). This could indicate that the timing and intensity of renal sexual segment hypertrophy can vary across a species' range. Geographic variation in reproductive cycles has been shown to occur in males of another wide-ranging snake, *Crotalus viridis* (Aldridge, 2002). Contrasts between *A. piscivorus*

populations could reveal interesting variation patterns that could be compared with species like *C. viridus*, to further understand the evolution of viperid mating systems.

Phylogenetic Considerations

As Sever et al. (2000) state in their work on the oviducal cycle of *Seminatrix pygaea*, phylogenetic implications based on the seasonal variation of oviducal morphology in squamates are not yet possible because of the lack of comparable studies. However, the current investigation which follows the "model system" presented by Sever et al. (2000), does shed new light on the subject of oviducal evolution, especially in the uterine and sperm storage regions of the oviduct. Unlike the viviparous colubrid investigated by Sever et al. (2000), the viviparous viperid (contained in a sister group to the Colubridae and Elapidae; Lawson et al., 2005) studied here still possesses highly secretory and specialized uterine glands. These glands are ultrastructurally similar to those of the oviparous lizard, *Saltuarius wyberba* (Girling et al., 1998). Uterine glands, responsible for egg-shell formation in squamates (Palmer et al., 1993), are thought to be highly reduced in viviparous squamates (for review see Blackburn, 1998). We hypothesize that although reduction in uterine gland number occurs with the shift from oviparity to viviparity, these glands become specialized for a viviparous mode of parity. Because viviparity has evolved numerous times in squamates (Blackburn, 1985; Shine, 1985), we propose that uterine glands in unrelated viviparous species of snakes may have evolved different specialized functions. This could indicate that reproductive morphology possesses phylogenetic importance. Previous studies on lizards support this hypothesis by revealing that lizards with slightly different forms of viviparity and oviparity possess unique uterine morphology (Girling et al., 1998; Girling, 2002; Thompson et al., 2006).

Unfortunately, gravid female *Agkistrodon piscivorus* were not attained in 2 years of collecting effort. Therefore, the ultrastructure of the gravid uterus of *A. piscivorus* has eluded this investigation. Histochemical work, however, on a gravid museum specimen shows that the tubular glands of the uterus are still very active with a protein secretion in late August (Siegel and Sever, 2008). Because the specimen was close to the time of parturition (Beyer, 1893; Conant, 1933; Allen and Swindell, 1948; Wharton, 1960; Funk, 1964; Burkett, 1966; Gloyd and Conant, 1990; Ford, 2002; Ford et al., 2004), and because even before ovulation these tubular glands are also actively secreting a protein product, it is conceivable that this activity spans the entire time of gestation. A similar secretory pattern occurs in lizards investigated with ultrastructural analysis (Girling et al., 1998).

Morphology of sperm storage tubules in the posterior infundibulum of *Agkistrodon piscivorus* is similar to that observed in all previous investigations on squamates, except one. Instead of the sperm storage tubules being just invaginations of the luminal epithelium of the posterior infundibulum like in *Seminatrix pygaea* (Sever and Ryan, 1999), sperm storage tubules of *A. piscivorus* are differentiated from the luminal border by a decreased number of ciliated cells when moving to the terminal end of the gland. This is similar to what has been found in ultrastructural studies of *Thamnophis sirtalis* (Hoffman and Wimsatt, 1972; Halpert et al., 1982). These sperm storage tubules possess a specialized PAS+ secretion during sperm presence, like that of other snakes (Hoffman and Wimsatt, 1972; Perkins and Palmer, 1996; Sever and Ryan, 1999), and sperm align themselves with their nuclei facing the distal secretory portion of the gland, which is consistent with the findings in other snakes (Hoffman and Wimsatt, 1972; Perkins and Palmer, 1996; for a review of sperm storage in squamates see Sever and Hamlett, 2002). Sperm in sperm storage tubules of *Seminatrix pygaea* align with their nuclei facing the lumen of the infundibulum (Sever and Ryan, 1999). It has been proposed that the ancestral location of sperm storage in snakes (and all squamates) is infundibular. The fact that all snakes investigated possess infundibular sperm storage (Sever and Hamlett, 2002) leads us to believe that sperm storage location might not be an adequate character to hypothesize phylogeny. However, ultrastructural investigations of sperm storage tubules have only been accomplished in the Viperidae and Colubridae (Hoffman and Wimsatt, 1972; Perkins and Palmer, 1996; Sever and Ryan, 1999; Siegel and Sever, 2008), and further investigation on sperm storage tubule ultrastructure could reveal further specializations in some taxa.

The vagina and anterior infundibulum exhibit different ultrastructural characteristics in female *Agkistrodon piscivorus* than those described in *Seminatrix pygaea* (Sever et al., 2000). The epithelium of the vagina in *S. pygaea* appears much less secretory, contains vaginal glands, and the majority of epithelial cells are ciliated instead of secretory (Sever et al., 2000). The vagina of *A. piscivorus*, in contrast, possesses no small glandular invaginations and is composed primarily of secretory cells. The vagina of *A. piscivorus* is enlarged and forms a vaginal pouch, common in viperids (Ludwig and Rahn, 1943). This "pouch" has not been observed in other families of snakes investigated and may be a synapomorphy for Viperidae. Considering that the vagina of *A. piscivorus* is completely different ultrastructurally than the vagina of the only other snake investigated with ultrastructural techniques (*S. pygaea*; Sever et al., 2000), comparison of this region is impossible. It is

yet to be determined if the vaginal pouch of viperids is even homologous with what has been termed the vagina of colubrids. The only noticeable difference in the anterior infundibulum of female *A. piscivorus* compared to that of *S. pygaea* is the presence of protein synthesis in *S. pygaea* (Sever et al., 2000). The infundibulum of *A. piscivorus* appears to function only in the synthesis of lipid material.

A mosaic of oviducal traits probably occurs in snakes because of the independent trajectories of oviducal evolution in different snakes. Currently it is not known which characters described earlier are derived or ancestral or if some of them are even homologous. Snakes have been diverging from a common ancestor for at least 140 million years (fossil record; White et al., 2005), and since then have branched into 19 families (European Molecular Biology Laboratory Database, 2006). Obviously, more work is needed on a greater variety of taxa to investigate phylogenetic implications of oviducal morphology in ophidians.

One recurring problem in studies on the snake oviduct concerns the divisions of the oviduct into specific regions and recognizing all distinct regions. The presence of a nonglandular uterus, "furrowed section of the uterus" (Halpert et al., 1982; Aldridge, 1992), or histologically unique anterior vagina (Ludwig and Rahn, 1943) is reported in only a few investigations, but is present in both colubrids and viperids (Ludwig and Rahn, 1943; Halpert et al., 1982; Aldridge, 1992; Siegel and Sever, 2006). In *Agkistrodon piscivorus*, this oviducal region is unique ultrastructurally from the vagina and glandular uterus. Because this area has not been thoroughly examined with the use of ultrastructural techniques in other snake families, comparisons are not possible. Great care must be taken in future research to look for these histologically unique regions so when literature on oviducal morphology in snakes increases, these regions can be properly compared.

ACKNOWLEDGMENTS

We thank Ryan Chabarria, Tiffany Schriever, and Devin Bloom for help in collecting *Agkistrodon piscivorus* specimens. This manuscript is part of a thesis submitted by the senior author to fulfill requirements for a Master of Science degree at Southeastern Louisiana University.

LITERATURE CITED

- Adams SM, Hosie MJ, Murphy CR, Thompson MB. 2004. Changes in oviducal morphology of the skink, *Lampropholis guichenoti*, associated with egg production. *J Morphol* 262: 536–544.
- Adams SM, Biazik J, Stewart RL, Murphy CR, Thompson MB. 2007a. Fundamentals of viviparity: comparison of seasonal changes in the uterine epithelium of oviparous and viviparous *Lerista bougainvillii* (Squamata:Scincidae). *J Morphol* 268: 624–635.
- Adams SM, Lui S, Jones SM, Thompson MB, Murphy CR. 2007b. Uterine epithelial changes during placentation in the viviparous skink *Eulamprus tympanum*. *J Morphol* 268:385–400.
- Aldridge RD. 1979. Seasonal spermatogenesis in sympatric *Crotalus viridis* and *Arizona elegans* in New Mexico. *J Herpetol* 13:187–192.
- Aldridge RD. 1992. Oviductal anatomy and seasonal sperm storage in the Southeastern Crowned Snake (*Tantilla coronata*). *Copeia* 1992:1103–1106.
- Aldridge RD. 1993. Male reproductive anatomy and seasonal occurrence of mating and combat behavior of the rattlesnake *Crotalus v. viridis*. *J Herpetol* 27:481–484.
- Aldridge RD. 2002. The link between mating season and male reproductive anatomy in the rattlesnakes *Crotalus viridis oreganus* and *Crotalus viridis helleri*. *J Herpetol* 36:295–300.
- Aldridge RD, Brown WS. 1995. Male reproductive cycle, age at maturity, and cost of reproduction in the rattlesnake (*Crotalus horridus*). *J Herpetol* 29:399–407.
- Aldridge RD, Duvall D. 2002. Evolution of the mating season in the pitvipers of North America. *Herpetological Monographs* 2002:1–25.
- Allen RE, Swindell D. 1948. Cottonmouth moccasin of Florida. *Herpetologica* 4:2–16.
- Barry FE, Weatherhead PJ, Phillipp DP. 1992. Multiple paternity in a wild population of northern water snakes *Nerodia sipedon*. *Behav Ecol Sociobiol* 30:193–199.
- Beyer GE. 1893. Contributions on the life histories of certain snakes. *Am Nat* XXXII:17–24.
- Birkhead TR, Møller AP, Sutherland WJ. 1993. Why do females make it so difficult for males to fertilize their eggs? *J Theor Biol* 161:51–60.
- Blackburn DG. 1985. Evolutionary origins of viviparity in the Reptilia. II. Serpentes, Amphisbaenia, and Echthysauria. *Amph Reptil* 5:259–291.
- Blackburn DG. 1998. Structure, function, and evolution of the oviducts of squamate reptiles, with special reference to viviparity and placentation. *J Exp Zool* 282:560–617.
- Bou-Resli MN, Bishay LF, Al-Zaid NS. 1981. Observations on the fine structure of the sperm storage crypts in the lizard *Acanthodactylus scutellatus hardyi*. *Arch Biol (Bruxelles)* 92:287–298.
- Bull JJ, Shine R. 1979. Iteroparous animals that skip opportunities for reproduction. *Am Nat* 114:296–303.
- Burkett RD. 1966. Natural history of the cottonmouth moccasin, *Agkistrodon piscivorus* (Reptilia). University of Kansas Publications, Museum of Natural History 1966:435–491.
- Conant R. 1933. Three generations of cottonmouths, *Agkistrodon piscivorus* (Lacepede). *Herpetol Rev* 1:43.
- Cuellar O. 1966. Oviducal anatomy and sperm storage structures in lizards. *J Morphol* 119:7–20.
- Diller LV, Wallace RL. 1984. Reproductive biology of the northern Pacific rattlesnake (*Crotalus viridis oreganus*) in northern Idaho. *Herpetologica* 40:182–193.
- European Molecular Biology Laboratory Reptile Database. 2006. Species number. <http://reptile-database.org>. Accessed June 2007.
- Ford NB. 2002. Ecology of the Western cottonmouth (*Agkistrodon piscivorus leucostoma*) in Northeastern Texas. In: Schuett GW, Höggren M, Douglas ME, Greene HW, editors. *Biology of the Vipers*. Utah: Eagle Mountain Publishing. pp 167–177.
- Ford NB, Brischoux F, Lancaster D. 2004. Reproduction in the western cottonmouth, *Agkistrodon piscivorus leucostoma*, in a floodplain forest. *SW Naturalist* 49:465–471.
- Funk RS. 1964. Birth of a brood of Western cottonmouths, *Agkistrodon piscivorus leucostoma*. *Trans Kans Acad Sci* 67:199.
- Gibson AR, Falls JB. 1975. Evidence for multiple insemination in the common garter snake, *Thamnophis sirtalis*. *Can J Zool* 53:1362–1368.

- Girling JE. 2002. The reptilian oviduct: A review of structure and function and directions for future research. *J Exp Zool* 293:141–170.
- Girling JE, Cree A, Guillelte LJ Jr. 1997. Oviductal structure in a viviparous New Zealand gecko, *Hoplodactylus maculatus*. *J Morphol* 234:51–68.
- Girling JE, Cree A, Guillelte LJ Jr. 1998. Oviductal structure in four species of gekkonid lizard differing in parity mode and eggshell structure. *Reprod Fertil Dev* 10:139–154.
- Gloyd HK, Conant R. 1990. Snakes of the *Agkistrodon* Complex: A monographic review. Society for the Study of Amphibians and Reptiles (SSAR), Contributions to Herpetology 6. Oxford, Ohio. pp 1–614.
- Graham S. 2006. An integrative analysis of reproduction and stress in free living male cottonmouths, *Agkistrodon piscivorus*, M.S. Thesis, Georgia State University, Atlanta. pp 1–85.
- Halpert AP, Garstka WR, Crews D. 1982. Sperm transport and storage and its relation to the annual cycle of the female red-sided garter snake, *Thamnophis sirtalis parietalis*. *J Morphol* 174:149–159.
- Hoffman LH, Wimsatt WA. 1972. Histochemical and electron microscopic observations on the sperm receptacles in the garter snake oviduct. *Amer J Anat* 134:71–96.
- Johnson LF, Jacob JS, Torrance P. 1982. Annual testicular and androgenic cycles of the cottonmouth (*Agkistrodon piscivorus*) in Alabama. *Herpetologica* 38:16–25.
- Lawson R, Slowinski JB, Crother BI, Burbrink FT. 2005. Phylogeny of the Colubroidea (Serpentes): New evidence from mitochondrial and nuclear genes. *Mol Phylo Evo* 37:581–601.
- Ludwig M, Rahn H. 1943. Sperm storage and copulatory adjustment in the prairie rattlesnake. *Copeia* 1943:15–18.
- Palmer BD, Demarco VG, Guillelte JR. 1993. Oviductal morphology and eggshell formation in the lizard, *Sceloporus woodi*. *J Morphol* 217:205–217.
- Perkins JM, Palmer BD. 1996. Histology and functional morphology of the oviduct of an oviparous snake, *Diadophis punctatus*. *J Morphol* 277:67–79.
- Rahn H. 1942. The reproductive cycle of the prairie rattlesnake. *Copeia* 1942:233–240.
- Sever DM, Ryan TJ. 1999. Ultrastructure of the reproductive system of the Black Swamp Snake (*Seminatrix pygaea*). I. Evidence for oviducal sperm storage. *J Morphol* 241:1–18.
- Sever DM, Hamlett WC. 2002. Female sperm storage in reptiles. *J Exp Zool* 292:187–199.
- Sever DM, Hopkins WA. 2004. Oviductal sperm storage in the Ground Skink *Scincella laterale* Holbrook (Reptilia: Scincidae). *J Exp Zool* 301A:599–611.
- Sever DM, Ryan TJ, Morris T, Patton D, Swafford S. 2000. Ultrastructure of the reproductive system of the Black Swamp Snake (*Seminatrix pygaea*). II. Annual oviducal cycle. *J Morphol* 245:146–160.
- Sever DM, Siegel DS, Bagwill A, Eckstut ME, Alexander L, Camus A, Morgan C. 2008. Renal sexual segment of the Cottonmouth Snake, *Agkistrodon piscivorus* (Reptilia, Squamata, Viperidae). *J Morphol* 269:640–653.
- Shine R. 1985. The evolution of viviparity in reptiles: An ecological analysis. In: Gans C, Billet F, editors. *Biology of the Reptilia*, Vol. 15. New York: Wiley. pp 605–694.
- Siegel DS, Sever DM. 2006. Utero-muscular twisting and sperm storage in viperids. *Herpetol Con Biol* 1:87–92.
- Siegel DS, Sever DM. 2008. Sperm aggregations in female *Agkistrodon piscivorus* (Reptilia:Squamata): A histological and ultrastructural investigation. *J Morphol* 269:189–206.
- Stille B, Madsen T, Niklasson M. 1986. Multiple paternity in the Adder, *Vipera berus*. *Oikos* 47:173–175.
- Stille B, Niklasson M. 1987. Within season multiple paternity in the Adder, *Vipera berus*: A reply. *Oikos* 49:232–233.
- Thompson MB, Biazik JB, Lui S, Adams SM, Murphy CR. 2006. Morphological and functional changes to the uterus of lizards with different placental complexities. *Herpetological Monographs* 20:178–185.
- Wharton CH. 1960. Birth and behavior of a brood of cottonmouths, *Agkistrodon piscivorus* with notes on tail luring. *Herpetologica* 16:125–129.
- Wharton CH. 1966. Reproduction and growth in the cottonmouths, *Agkistrodon piscivorus* Lacépède, of Cedar Keys, Florida. *Copeia* 1966:149–161.
- White ME, Kelly-Smith M, Crother BI. 2005. Higher-level snake phylogeny as inferred from 28S ribosomal DNA and morphology. In: Donnelly MA, Crother BI, Guyer C, Wake MH, White ME, editors. *Ecology and evolution in the tropics*. Illinois: The University of Chicago Press. pp 156–177.