

Ultrastructure of the Reproductive System of the Black Swamp Snake (*Seminatrix pygaea*): Part I. Evidence for Oviducal Sperm Storage

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ABSTRACT Oviducal sperm storage in the viviparous (lecithotrophic) colubrid snake *Seminatrix pygaea* was studied by light and electron microscopy. Out of 17 adult snakes examined from May–October, sperm were found in the oviducts of only two specimens. In a preovulatory female sacrificed 14 May, sperm were found in the oviducal lumen and sperm storage tubules (SSTs) of the posterior infundibulum. In a nonvitellogenic female sacrificed 9 June, sperm were found in the lumen and glands of the posterior uterus and anterior vagina, indicating a recent mating. The glands in the posterior infundibulum and vagina were simple or compound tubular, whereas glands in the uterus always were simple tubular. The epithelium of the sperm storage glands was not modified from that lining the rest of the oviduct. The cuboidal or columnar epithelium consisted of alternating ciliated and secretory areas. The secretory product released into the lumen by a merocrine process contained mucoprotein. Lipid droplets also were numerous in the epithelium. Portions of sperm sometimes were embedded in the apical cytoplasm or in secretory material. A carrier matrix containing a mucoid substance, desquamated epithelium, lipids, membranous structures, and possibly phagocytes was found around sperm in the posterior uterus. *J. Morphol.* 241:1–18, 1999. © 1999 Wiley-Liss, Inc.

KEY WORDS: Reptilia; Serpentes; *Seminatrix pygaea*; ultrastructure; oviduct; sperm

Sperm storage tubules (SSTs) probably occur in the oviducts of all female snakes (Fox and Dessauer, '62; Devine, '84), and sperm storage in the female oviduct also has been reported in the other extant reptile groups, except Rhynchocephalia (for reviews see Howarth, '74; Saint-Girons, '75, '82; Fox, '77; Devine, '84; Gist and Jones, '87; Birkhead and Moller, '93; Blackburn, '98). The reptilian oviduct basically consists of an anterior infundibulum, into which eggs are ovulated from the ovary; a middle uterus where eggs are held until oviposition or embryos develop until parturition; and a posterior vagina that opens into the cloaca (Gist and Jones, '87; Blackburn, '98). In addition, turtles and crocodylians have a "tubal" region between the infundibulum and uterus where albumen-secreting glands, absent in snakes and lizards, are found. The site of sperm storage in the oviduct of crocodylians

is unknown (Davenport, '95), but in turtles SSTs occur in the tubal region (Gist and Jones, '87). In lizards and snakes, SSTs occur at the junction of the infundibulum and uterus, and sperm also may be stored in furrows or glands in the vaginal mucosa (Fox, '56; Cuellar, '66; Halpert et al., '82).

Detailed anatomical studies on the histology and cytology of the sperm storage regions of the oviduct in reptiles are few. In snakes, studies at the light microscopy level exist (chronologically) for *Thamnophis sirtalis* (Rahn, '40; Fox, '56; Hoffman and Wimsatt, '72; Halpert et al., '82), *Crotalus viridis*

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(Ludwig and Rahn, '43), *T. elegans* (W. Fox, '56), *Vipera aspis* (Saint Girons, '57), *Typhlops* and *Leptotyphlops* (Fox and Dessauer, '62), and *Tantilla coronata* (Aldridge, '92). Perkins and Palmer ('96) used both light microscopy and scanning electron microscopy (SEM) to study oviducal cycles of sperm storage in *Diadophis punctatus*. The only study prior to the present one to utilize transmission electron microscopy (TEM) to study the ultrastructure of sperm storage in a female snake was by Hoffman and Wimsatt ('82) on *T. sirtalis*.

In this article, we report on the histology and cytology of sperm storage in the oviduct of the black swamp snake, *Seminatrix pygaea*, in South Carolina. Other aspects of the reproductive cycle of the population we studied were described by Seigel et al. ('95). This species is a small (adults 20–40 cm snout-vent length, SVL), highly aquatic, natricine snake found along the Atlantic coastal plain in the southeastern United States (Conant and Collins, '98; Dorcas et al., '98). *S. pygaea* is viviparous by lecithotrophy, i.e., most if not all nutrients for fetal development are supplied via the yolk of the ovulated ovum (Blackburn, '98). Our study is the first to use a combination of light microscopy, SEM, and TEM to describe sperm in the oviduct of a snake or any other reptile. Additional reports on the ultrastructure of the reproductive system of *S. pygaea* will describe the entire oviduct through its annual cycle (Part II), fetal/maternal rela-

tionships (Part III), and the male reproductive system (Part IV).

MATERIALS AND METHODS

A total of 17 female *Seminatrix pygaea* were collected at Ellenton Bay, located on the Department of Energy's Savannah River Site in Aiken County, South Carolina. Ellenton Bay is a shallow (2 m maximum depth), 10 ha "Carolina bay" that is relatively permanent (Gibbons and Semlitsch, '91).

Collections were made during four periods in 1998: 6–7 May, 1–5 June, 22–24 July, and 29 September–2 October, and specimens were sacrificed within 10 days of capture (Table 1). Specimens were collected in unbaited minnow traps and from under cover boards alongside the bay. Since the reptiles, and in particular the *Seminatrix* population, of Ellenton Bay are the subjects of long-term monitoring studies, we sought to minimize the number of specimens removed from the population. Specimens were killed by a lethal injection (3–5 ml) of Nembutal (Abbott Laboratories, North Chicago, IL). Carcasses of all specimens were preserved in 10% neutral buffered formalin (NBF) and housed in the research collections at Saint Mary's College.

After sacrifice, SVL was measured to the nearest mm. The cloaca and both oviducts were removed in their entirety and fixed in a 1:1 solution of 2.5% glutaraldehyde in Millonig's phosphate buffer at pH 7.4 and 3.7%

TABLE 1. Specimens used in this study

| Date sacrificed | SVL (cm) | Location | Follicles/eggs | | | |
|-----------------|----------|----------|----------------|-----------|------|------|
| | | | N | Range | Mean | SE |
| 14 May 98 | 23.7 | Ovaries | 6R 6L | 1.8–3.1 | 2.4 | 0.11 |
| | 28.0 | Ovaries | 5R 3L | 5.0–7.2 | 5.8 | 0.35 |
| | 32.0 | Ovaries | 10R 7L | 1.8–3.5 | 2.7 | 0.12 |
| 9 June | 33.0 | Ovaries | 5R 4L | 7.0–11.2 | 9.6 | 0.47 |
| | 26.0 | Uteri | 3R 2L | 7.4–11.2 | 9.2 | 0.78 |
| | 29.0 | Uteri | 3R 1L | 17.5–18.2 | 17.9 | 0.14 |
| | 30.2 | Uteri | 5R 3L | 13.0–15.3 | 13.9 | 0.31 |
| | 35.5 | Ovaries | 5R 5L | 2.0–3.6 | 2.7 | 0.14 |
| 30 July | 29.0 | Ovaries | 7R 3L | 1.7–2.2 | 2.0 | 0.05 |
| | | Uteri | 2R 2L* | | | |
| | 27.5 | Ovaries | 6R 5L | 1.8–2.6 | 2.2 | 0.08 |
| | 27.5 | Ovaries | 6R 4L | 1.8–2.3 | 2.0 | 0.06 |
| 8 October | 24.0 | Ovaries | 5R 3L | 1.4–2.0 | 1.7 | 0.06 |
| | 26.0 | Ovaries | 7R 7L | 2.0–3.8 | 2.9 | 0.14 |
| | 29.1 | Ovaries | 7R 9L | 1.5–3.0 | 2.2 | 0.11 |
| | 30.1 | Ovaries | 13R 10L | 1.2–3.3 | 2.4 | 0.12 |
| | 33.0 | Ovaries | 11R 7L | 1.5–2.6 | 2.1 | 0.08 |
| | 37.4 | Ovaries | 14R 11L | 1.8–3.6 | 2.7 | 0.10 |

*Four fully formed fetuses, two of which were preserved in situ; other two measured 95 mm and 97 mm SVL.

formaldehyde buffered to pH 7.2 with monobasic and dibasic phosphate.

Following the criteria of Blackburn ('98), three basic divisions of the oviduct could be recognized grossly: infundibulum (with a posterior SST area), uterus, and vagina (Fig. 1). For electron microscopy, sections of the oviduct 2 mm in length were removed from each division of the right oviduct. Tissue was taken from the anterior portion of the infundibulum as well as the SST area. From the uterine area, tissue was taken from both the anterior and posterior ends of the uterus in nongravid snakes, whereas in gravid snakes, tissue was taken from the uterine lining surrounding an embryo and from the inter-embryonic area between eggs (Fig. 1B). After initial fixation, tissues were rinsed in water, postfixed in 2% osmium tetroxide, and dehydrated through a graded series of ethanol.

For SEM, the tissue was then subjected to critical point drying, mounted on a metal stub with adhesive tape, and sputter-coated with gold in a Denton Desk II (Denton Vacuum, Moorestown, NJ). The specimens were examined with a JEOL JSM-T300 scanning electron microscope (JEOL USA, Peabody, MA).

For TEM, after dehydration tissues were cleared in propylene oxide and immersed in increasing concentrations of an epoxy resin (EmBed 812, Electron Microscopy Sciences, Fort Washington, PA) in absolute ethanol before polymerization in pure resin for 36 h at 60°C. Plastic sections were cut with an RMC MT7 ultramicrotome (Research and Manufacturing Co., Tucson, AZ) and DiATOME diamond knives (DiATOME, Biel, Switzerland). Semithin sections (0.5 μ m) for light microscopy were placed on microscope slides and stained with toluidine blue. Ultrathin sections (70 nm) were collected on uncoated copper grids and stained with solutions of uranyl acetate and lead citrate. Ultrathin sections were viewed with a Hitachi H-300 transmission electron microscope (Nissei Sangyo America, Mountain View, CA).

The entire left oviduct was prepared for light microscopy by the standard paraffin method. Following fixation in the 1:1 glutaraldehyde:formalin solution used for electron microscopy, the oviduct was cut into smaller portions, dehydrated in ethanol, cleared in HistoSol (National Diagnostics, NJ), and em-

bedded in paraffin. Sagittal sections were cut at 10 μ m and placed on albuminized slides. Alternate slides were stained with hematoxylin-eosin (general cytology), Alcian blue 8GX at pH 2.5 (primarily carboxylated glycosaminoglycans) counterstained with periodic acid and Schiff's reagent (neutral carbohydrates), brilliant indocyanine 6B (proteins), and Cason's trichrome (connective tissue). Staining procedures followed Kieran ('90).

Permission to make collections was granted by the administration of the Savannah River Ecology Laboratory. Protocols were approved by the Animal Care and Use Committee at Saint Mary's College.

RESULTS

Female reproductive cycle

Vitellogenesis was evident in two of the four females collected during May, in which follicles were 5.0–11.2 mm in mean diameter (Table 1). Ovulation had occurred in reproducing females collected in June, and eggs in the uteri were 7.4–18.2 mm mean diameter. Parturition likely occurs in late July and August. One female sacrificed 30 July had four fully developed fetuses, two of which measured 95 mm (male) and 97 mm (female) SVL, approximating the mean size for newly born males (115.8 mm) and females (114.8 mm) of *Seminatrix pygaea* (Seigel et al., '95). The five females collected in late September and early October contained nonvitellogenic follicles 2.1–2.9 mm mean diameter.

Oviducal sperm

Sperm were found in the oviducal lumen and in exocrine glands, SSTs, of the posterior infundibulum in one of two vitellogenic, preovulatory females collected in May (Fig. 2A). Sperm were lacking in other regions of the oviduct in this specimen. Sperm also were found in glands and in the lumen of the vagina (Fig. 2B) and posterior uterus (Fig. 2C) in one of three nonvitellogenic, nonreproducing females collected in June. Sperm in the posterior uterus mostly were associated with a carrier matrix, as described at the ultrastructural level later in this report. More anterior regions of the oviduct in this specimen lacked sperm and had glands that were narrow and constricted or, conversely, occluded with secretory material (Fig. 2D). All other females examined lack sperm anywhere in the oviduct.

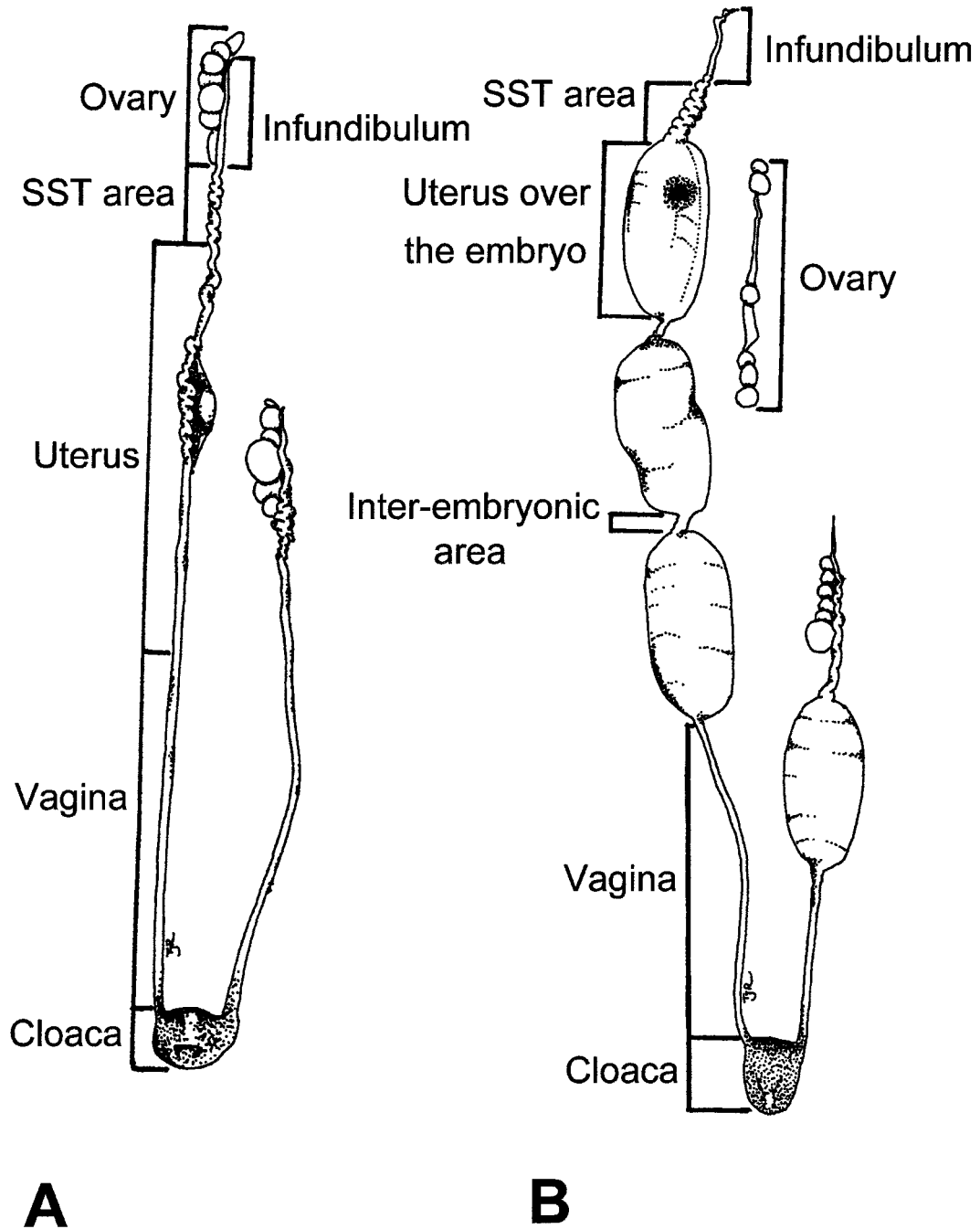


Fig. 1. Drawing of oviducts of (A) a 32 cm SVL nonvitellogenic female *Seminatrix pygaea* collected and sacrificed in May and (B) a 29.0 cm SVL gravid female collected and sacrificed in June.

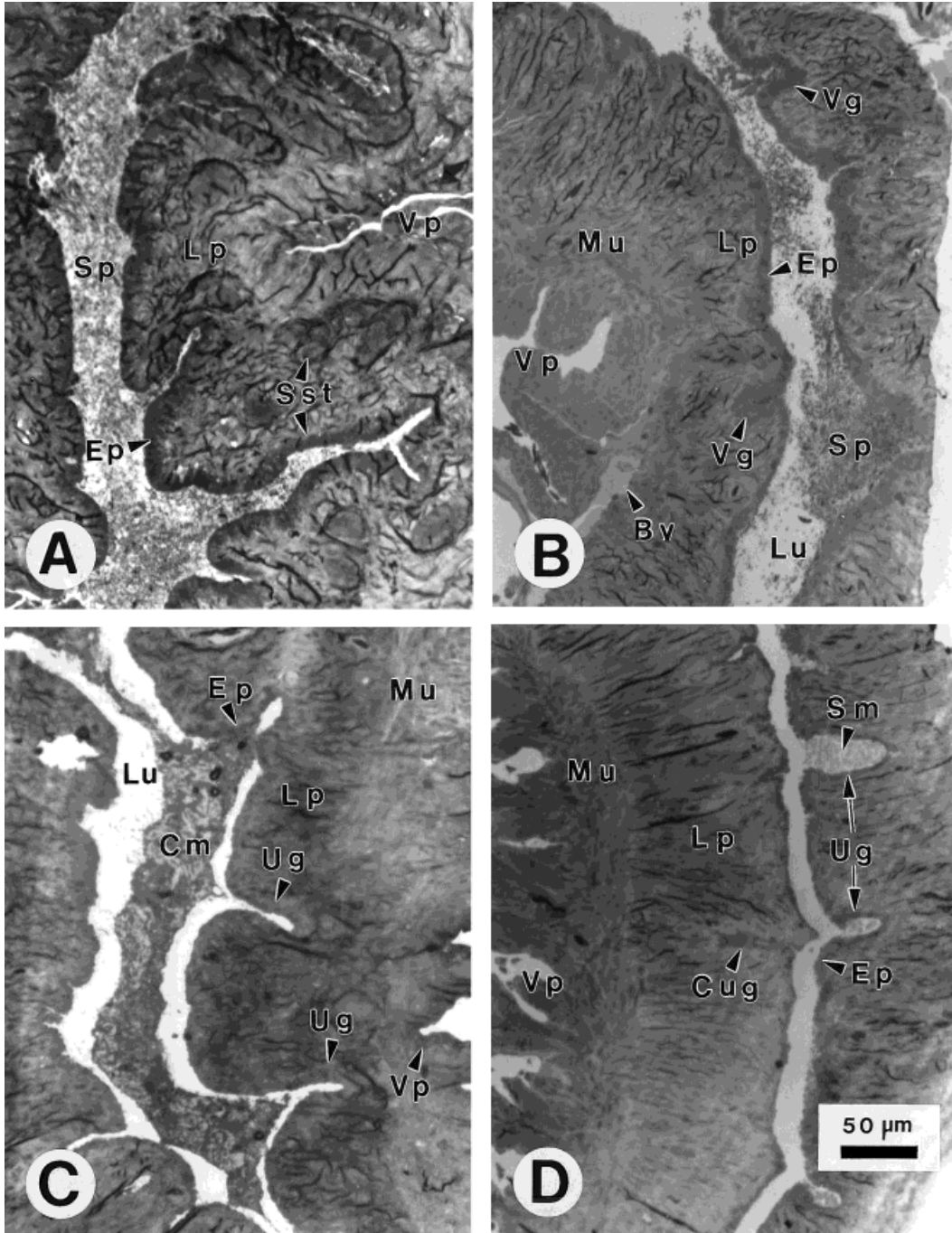


Fig. 2. Sagittal semithin sections stained with toluidine blue through the oviducts of *Seminatrix pygaea*. **A**: Posterior infundibulum of a 33 cm SVL preovulatory female collected and sacrificed in May with abundant sperm in the oviducal lumen and sperm storage tubules (Sst) in this region. (**B–D**) Nonvitellogenic female 35.5 cm SVL collected and sacrificed in June with sperm in

the anterior vagina and posterior uterus. **B**: Vagina. **C**: Posterior uterus. **D**: Anterior uterus. Bv, blood vessel; Cm, carrier matrix; Cug, constricted uterine gland; Ep, epithelium; Lp, lamina propria; Lu, lumen; Mu, muscularis; Sp, sperm; Sst, sperm storage tubule; Ug, uterine gland; Vg, vaginal gland; Vp, visceral pleuroperitoneum.

SST area

Distribution of sperm in a portion of the SST region is illustrated by SEM in Figure 3. Aggregations of sperm can occur in folds and in the SSTs, and sperm are also scattered in the lumen (Fig. 3A). Sperm are not found in orderly arrays but tend to form tangled masses when clustered in folds of the oviduct and are often associated with aggregates of membranous structures (Fig. 3B). No consistent relationship was observed between orientation of sperm and secretory or ciliated portions of the oviducal lining (Fig. 3C).

Ultrastructure of the SST area is illustrated with TEM in Figures 4–5. The epithelium is simple and varies from cuboidal to columnar (Fig. 4A). The epithelium consists mostly of “dark” cells with elongate heterochromatic nuclei aligned with the long axis of the cell and occupying much of the cytoplasm (Fig. 4A). Occasional “light cells” apparently undergoing degenerative processes occur among the dark cells. The epithelium consists of alternating regions of ciliated and nonciliated secretory cells (Fig. 4A,B). The secretory cells typically possess microvilli (Fig. 4B). Both ciliated and nonciliated cells contain abundant lipid droplets, especially basally (Fig. 4A). In addition, the secretory cells contain apical secretory vacuoles consisting of an electron-dense particle surrounded by flocculent material (Fig. 4B).

The secretory areas of the epithelium containing these vacuoles are strongly PAS+ (neutral carbohydrates), moderately positive with brilliant indocyanine 6B (proteins), and do not react, or react weakly, with Alcian blue 8GX at pH 2.5 (carboxylated glycosaminoglycans). Thus, the product appears to contain mucoprotein. Release of the substance apparently is by a merocrine process, as the unit membranes of the vacuoles appear to fuse with the plasma membranes of the epithelial oviducal border (arrowheads, Fig. 4B).

Deep to the epithelium is a thick sheath of collagen (Fig. 4A), which is highly vascular. No myoepithelium or contractile elements are associated with the basal border of the oviducal epithelium. As noted with SEM (Fig. 3A,B), sperm observed by TEM are scattered in the oviducal lumen of the SST area, with no orderly arrangement (Fig. 4A,C). The sperm appear normal in cytology and are in a matrix of membranous structures, perhaps resulting from the breakdown of secre-

tory products (Fig. 4B,C). Occasionally, a sperm nucleus is found embedded in the apical cytoplasm of a secretory cell, but no evidence of sperm degradation was observed (Fig. 4B).

SSTs are simple or occasionally branched invaginations of the oviducal lining (Figs. 3A, 5A–C). SSTs are tubular and lack enlarged distal acini (Fig. 5B,C). The glands did not differ cytologically from the lining of the oviducal lumen, and possess areas of ciliated and secretory cells. Alignment of sperm was somewhat more orderly than in the oviducal lumen, with sperm tails predominating in the proximal ends of the glands (Fig. 5A) and sperm heads in the distal ends of the glands (Fig. 5B,C). As in the oviducal lining proper, occasional light cells, exhibiting indications of degradation, are found among dark cells (Fig. 5B). The release of mucoprotein from the SSTs also appears to be merocrine (arrowheads, Fig. 5D).

Other ultrastructural features associated with the linings of the oviduct and of the SSTs are rather typical for the epithelium of metabolically active mucous membranes. Inter-cellular canaliculi between epithelial cells are narrow and labyrinthine (Fig. 4B), and tight junctions occur at the luminal border (Fig. 5C). Clusters of elongate mitochondria occur in both ciliated and nonciliated cells (Fig. 5D). Golgi complexes with budding condensing vacuoles that will mature into the secretory vacuoles are common in the supranuclear cytoplasm (as illustrated later for vaginal glands). Microfilaments involved in movement of secretory products are associated with the vacuoles.

Anterior vagina

As in the SST area, sperm in the vagina are found in disorderly clusters in the vaginal lumen (Figs. 2B, 6A). Desquamated portions of epithelial cells from the oviducal lining are associated with luminal sperm (Fig. 6B). Whether desquamation results from mechanical injury during mating or represents some apocrine or even holocrine secretory process could not be determined. Sperm in the lumen appear normal in cytology and, like sperm in the SST area, are in a matrix of membranous structures (Fig. 6C).

The vaginal lining and the vaginal glands are virtually identical to those in the SST area (e.g., Figs. 5, 7). The same arrangement of alternating ciliated and nonciliated secretory cells occurs, and the epithelium of the

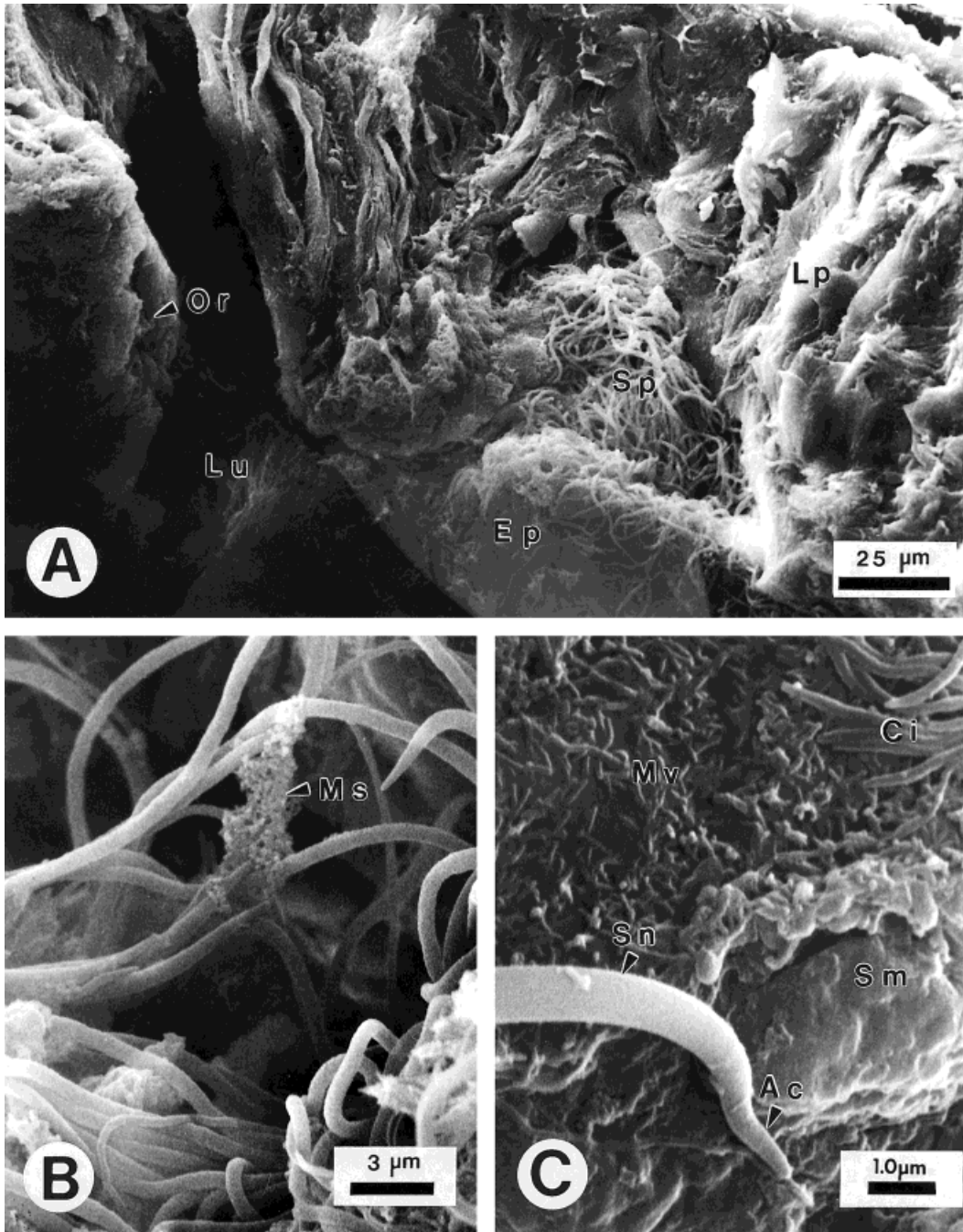


Fig. 3. Scanning electron micrographs of the posterior infundibulum of a 33.0 cm SVL preovulatory *Seminatrix pygaea* collected and sacrificed in May. **A:** Lumen of the oviduct and associated sperm. The sperm are in a fold of the oviduct, not in a sperm storage tubule. **B:** Tangle of sperm on the epithelial surface of the oviduct.

C: Sperm head in relation to secretory material and cilia. Ac, acrosome; Ci, cilia; Ep, epithelium; Lp, lamina propria; Lu, lumen; Ms, membranous structures; Mv, microvilli; Or, orifice of a sperm storage tubule; Sm, secretory material; Sn, sperm nucleus.

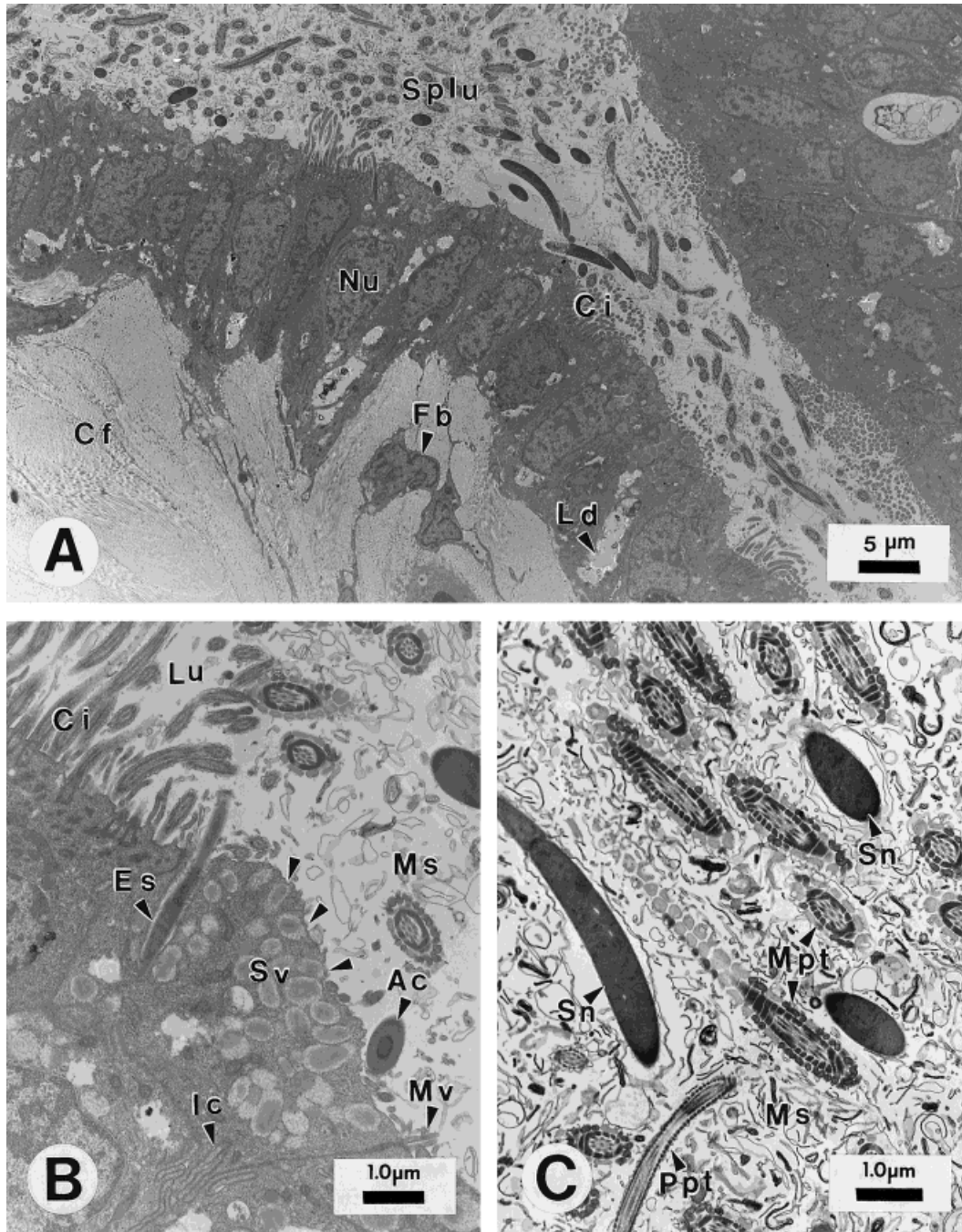


Fig. 4. Transmission electron micrographs of the posterior infundibulum of a 33.0 cm SVL preovulatory *Semimatrix pygaea* collected and sacrificed in May. **A:** Oviducal mucosa and adjacent lumen. **B:** Apical border of oviducal epithelium. Unlabeled arrowheads indicate areas of membrane fusion resulting in a merocrine secretory process. **C:** Sperm in the lumen of the oviduct. Ac,

acrosome; Cf, collagen fibers; Ci, cilia; Es, embedded sperm; Fb, fibroblast; Ic, intercellular canaliculus; Ld, lipid droplet; Mpt, middle piece of the tail; Ms, membranous structures; Mv, microvilli; Nu, nucleus of an epithelial cell; Ppt, principle piece of the tail; Sn, sperm nucleus; Sv, secretory vacuoles.

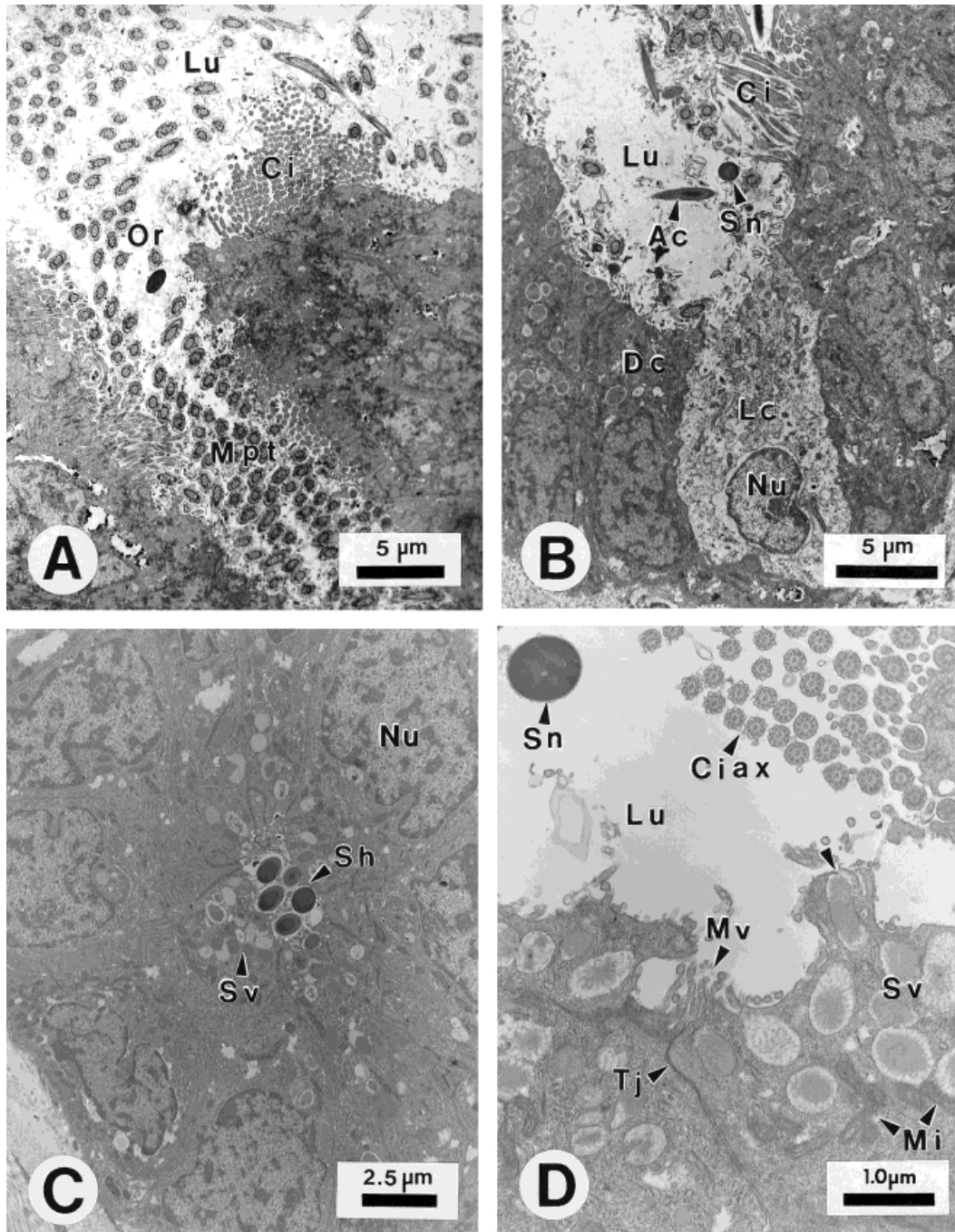


Fig. 5. Transmission electron micrographs of the posterior infundibulum of a 33.0 cm SVL preovulatory *Seminatrix pygaea* collected and sacrificed in May. **A:** Orifice of a sperm storage tubule. **(B–C)** Distal ends of sperm storage tubules. **D:** Apical epithelial border of a sperm storage tubule. Unlabeled arrowheads indicate mero-

crine release of secretory material. Ac, acrosome; Ci, cilia; Ci, cilia; Ci, cilia axonemes; Dc, dark cell; Lc, light cell; Lu, lumen; Mi, mitochondria; Mpt, middle piece of the tail; Mv, microvilli; Nu, nucleus of an epithelial cell; Or, orifice of a sperm storage tubule; Sh, sperm heads; Sn, sperm nucleus; Sv, secretory vacuoles; Tj, tight junction.

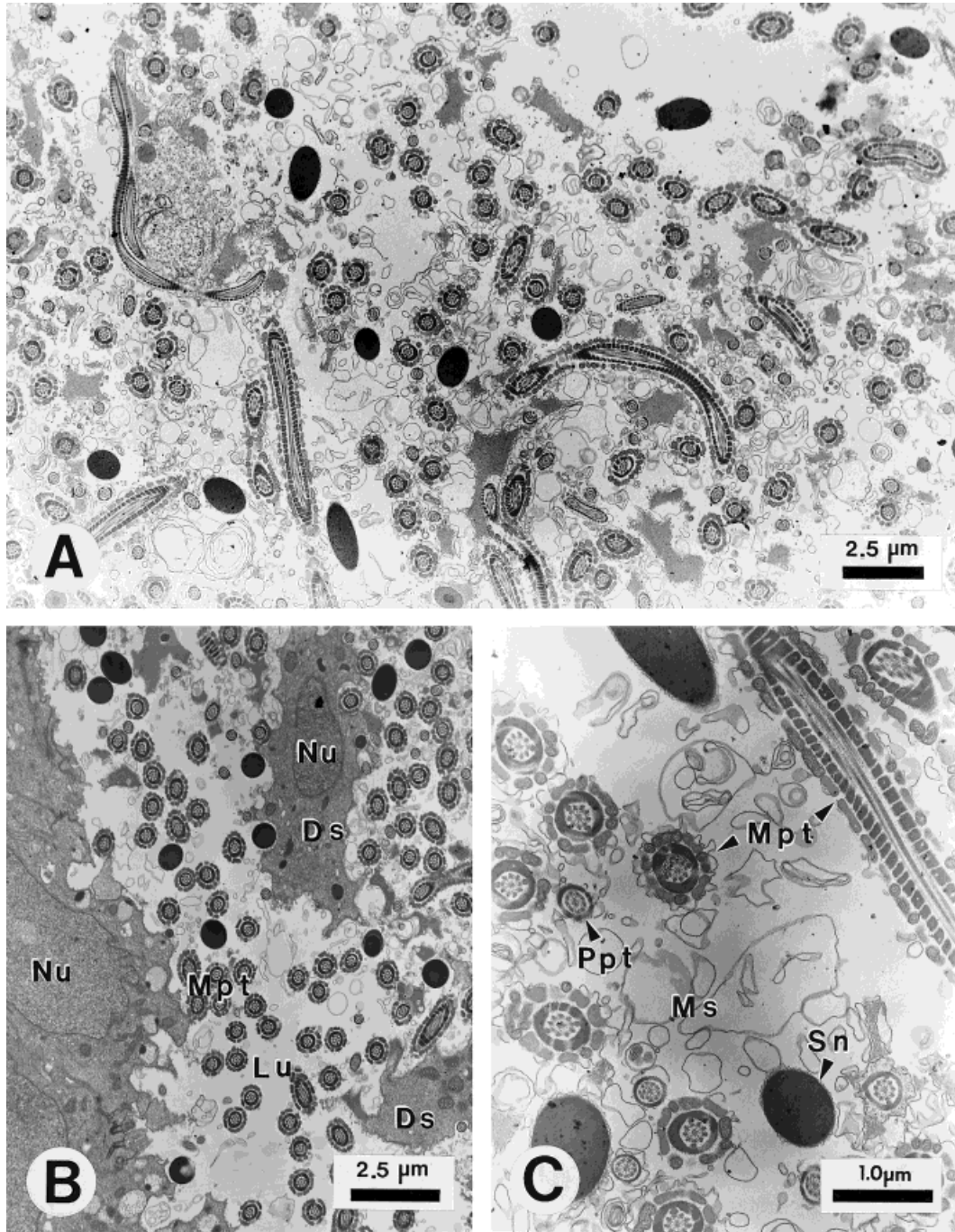


Fig. 6. Transmission electron micrographs of the vagina of a 35.5 cm SVL nonvitellogenic *Seminatrix pygaea* collected and sacrificed in June. **A:** Sperm in the lumen of the vagina. **B:** Epithelial border adjacent to vaginal lumen. **C:** Detail of sperm in the lumen. Ds, desqua-

mated epithelial cell; Lu, lumen; Mpt, middle piece of the tail; Ms, membranous structures; Nu, nucleus of an epithelial cell; Ppt, principle piece of the tail; Sn, sperm nucleus.

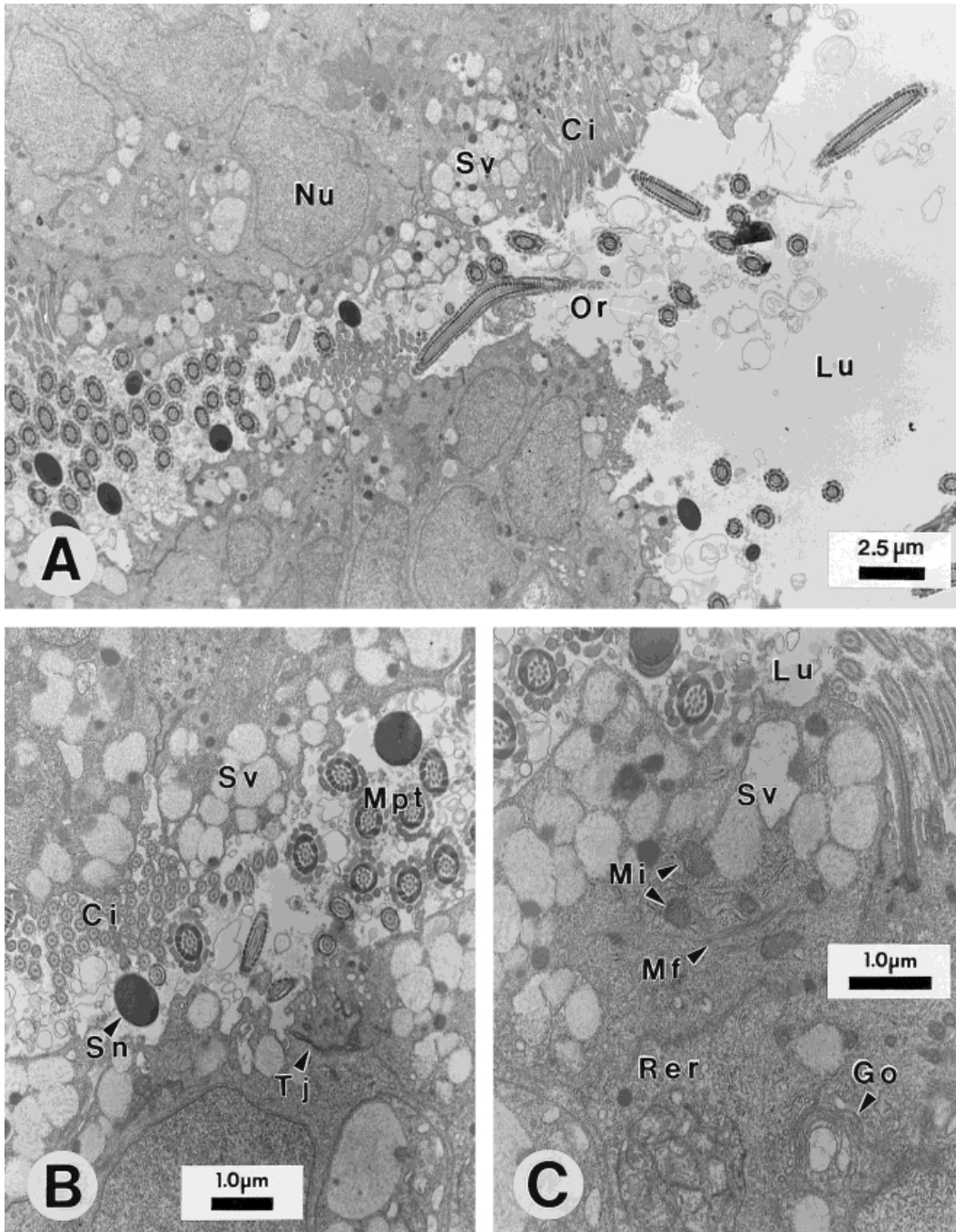


Fig. 7. Transmission electron micrographs of the vagina of a 35.5 cm SVL nonvitellogenic *Seminatrix pygaea* collected and sacrificed in June. **A:** Orifice of a vaginal gland and adjacent vaginal lining and lumen. **B:** Distal portions of a vaginal gland. **C:** Detail of apical cytoplasm of a vaginal gland. Ci, cilia; Go, Golgi com-

plex; Lu, lumen; Mf, microfilaments; Mi, mitochondria; Mpt, middle piece of the tail; Nu, nucleus of an epithelial cell; Or, orifice of a vaginal gland; Rer, rough endoplasmic reticulum; Sn, sperm nucleus; Sv, secretory vacuoles; Tj, tight junction.

vaginal glands does not differ cytologically from that of other regions of the anterior vagina (Fig. 7A,B). Sperm are aligned more orderly in the vaginal glands than the lumen of the vagina, with nuclei predominating in distal regions of the glands. Three differences occur between the epithelium in the SST region and the vagina. First, the vaginal nuclei are euchromatic (Fig. 7), whereas those in the SST area are heterochromatic (Fig. 5). Second, the secretory vacuoles of the vaginal epithelium mostly contain the flocculent material; when present, the dense particle is more eccentric (e.g., Figs. 5D, 7B,C). Finally, the epithelium reacts positively to both PAS and Alcian blue 8GX at pH 2.5, whereas the Alcian blue reaction was weak or absent in the infundibulum. Golgi complexes, rough endoplasmic reticulum (Rer), and microfilaments are associated with the secretory process (Fig. 7C).

Posterior uterus

The posterior uterus of the specimen possessing sperm in this region is characterized by an extraordinary luminal matrix-containing sperm (Fig. 8). This substance appears to be the "carrier matrix" described in the posterior oviduct of recently mated *Thamnophis sirtalis* by Halpert et al. ('82). The matrix is not uniform (Fig. 8A). The matrix consists primarily of a dense colloid of secretory material, but also contains desquamated epithelial cells and membranous structures (Fig. 8B,C). Some large spherical structures are composed of aggregations of membranous structures, lipid droplets, and other debris (Fig. 8C).

At least some of the matrix is secreted by the epithelial lining and uterine glands in this area (Figs. 2C, 9). The colloid of the carrier matrix was often observed in contact with the luminal border of secretory cells, and the vacuoles in these areas are flattened and composed primarily of flocculent material (Fig. 9A,C). Once again, the release of secretory material appears to be merocrine (Fig. 9C).

Secretory vacuoles are not numerous in the area of the carrier matrix, and their scarcity probably results from release of their products into the matrix (Fig. 9C). The matrix is extensive, occupying a considerable volume of the lumen (Figs. 2C, 8, 9). Secretions from the uterine epithelium and glands do not seem sufficient to account for the magnitude of the matrix, and the substances within the epithelial secretory vacuoles have

a different density than the colloid of the matrix (Fig. 9A,C). The matrix therefore also may contain contributions from male secretory products, such as seminal fluid from the sexual segment of the kidney or remnants of a copulatory plug.

Some of the unattached cells in the lumen of the vagina (Fig. 6B) and uterus (Fig. 8A,C) contain portions of sperm cells embedded in their cytoplasm. These cells may not represent desquamated (and deteriorating) oviducal epithelial cells, but rather a type of phagocyte.

The epithelium of the uterus does not differ histochemically from that of the vagina, and the carrier matrix contains a mixture of PAS and Alcian blue-positive material. The uterine lining and glands in this region do not differ histologically or cytologically from those of the vagina, except that the uterine glands are always simple tubular and do not branch (Fig. 2C). Figure 9B illustrates release of lipid material into the lumen; the uterus is the only region in which secretion of lipid was observed.

DISCUSSION

A number of articles have reviewed sperm storage in reptiles in general (Howarth, '74; Saint-Girons, '75; Fox, '77; Devine, '84; Gist and Jones, '87; Birkhead and Moller, '93) or particular taxa (lizards, Cuellar, '66; pit vipers, Schuett, '92; squamates, Blackburn, '98; turtles, Gist and Congdon, '98). Other articles have been concerned with records of sperm storage in various captive species, and are mostly anecdotal (Haines, '40; Stewart, '72; Magnusson, '79). The physiology of mating and oviducal cycles has received relatively little attention (Crews, '84; Whittier and Crews, '86), but much interest has focused on the relationship of mating and sperm storage to multiple paternity (Blanchard and Blanchard, '40; Blanchard, '42; Gibson and Falls, '75; Zweifel and Dessauer, '83; Schuett and Gillingham, '86; Schwartz et al., '89; Hoggren and Tegelstrom, '95) and sperm competition (Devine, '84; Birkhead and Moller, '93; Olsson and Madsen, '98). No review has focused strictly on the anatomy of oviducal sperm storage in snakes, so the following synopsis of past literature is presented.

Previous work

The first observations on the presence of living sperm in the oviducts of snakes did

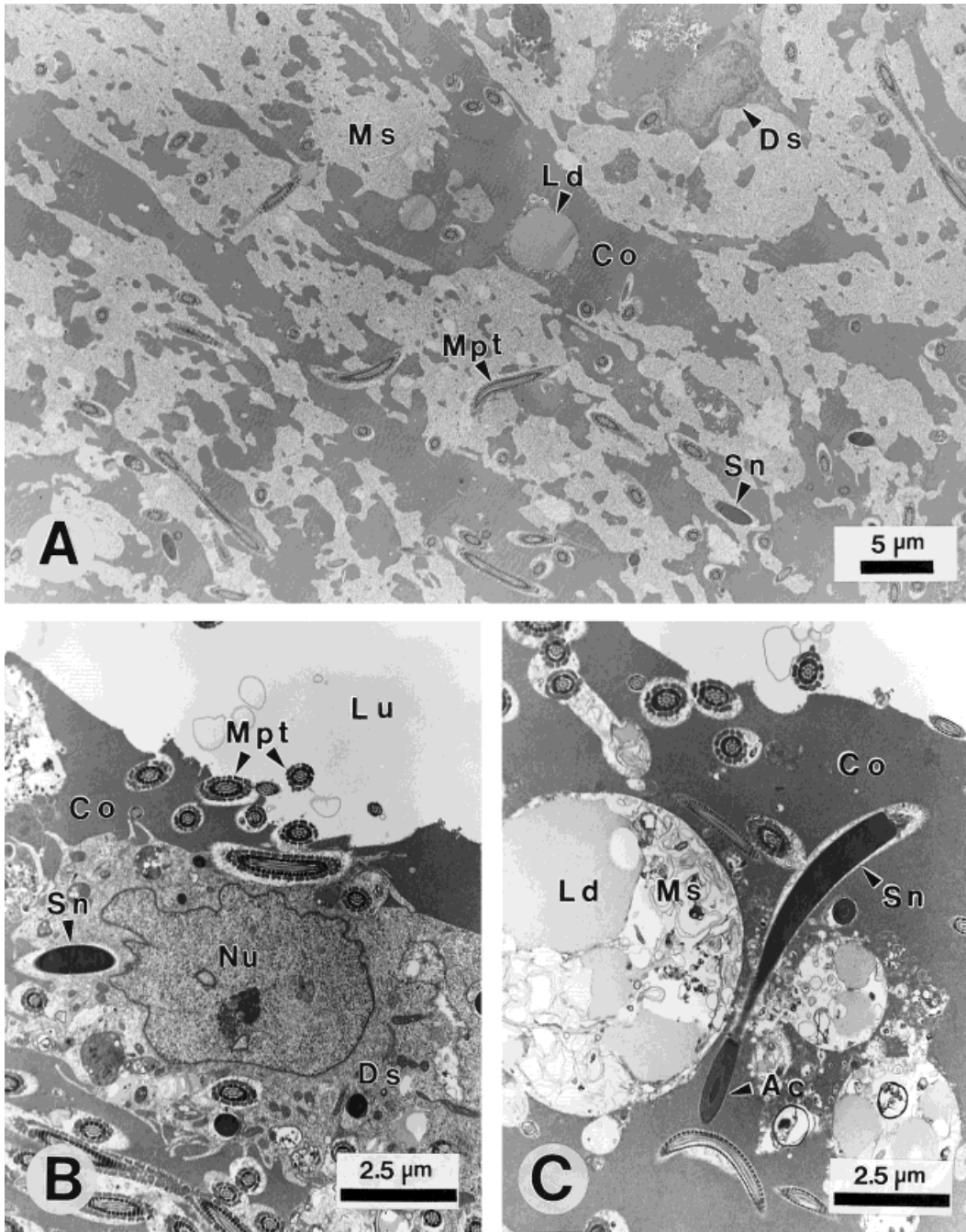


Fig. 8. Transmission electron micrographs of the posterior uterus of a 35.5 cm SVL nonvitellogenic *Seminatrix pygaea* collected and sacrificed in June. **A:** Overview of the carrier matrix and sperm in the lumen of the uterus. **B:** Desquamated epithelium in the carrier matrix. **C:** Lipid droplets and membranous structures in

the carrier matrix. Ac, acrosome; Co, colloid of the carrier matrix; Ds, desquamated epithelial cell; Ld, lipid droplet; Lu, lumen; Mpt, middle piece of the tail; Ms, membranous structures; Nu, nucleus of an epithelial cell; Sn, sperm nucleus.

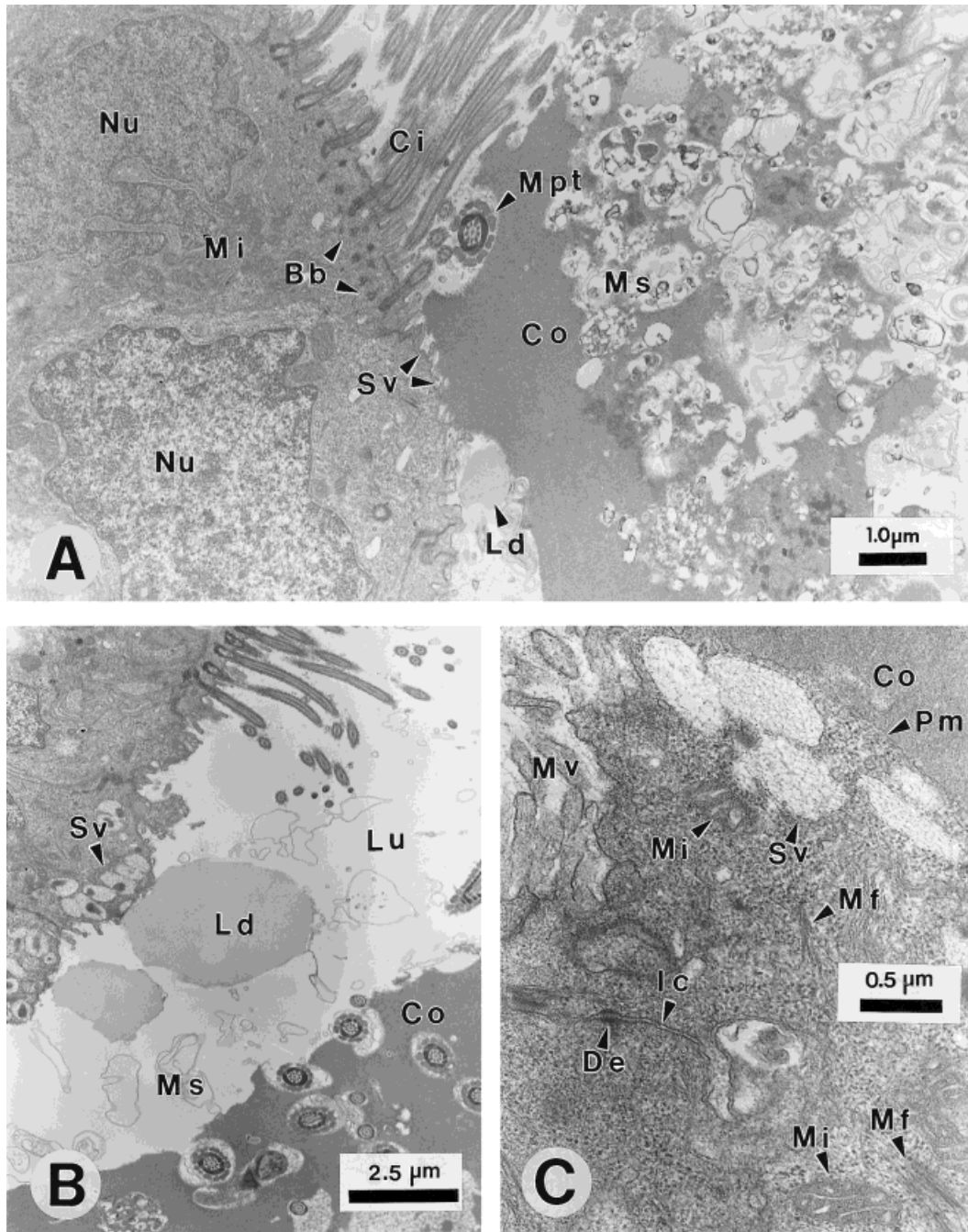


Fig. 9. Transmission electron micrographs of the posterior uterus of a 35.5 cm SVL nonvitellogenic *Seminatrix pygaea* collected and sacrificed in June, illustrating aspects of carrier matrix formation. **A:** Carrier matrix in relation to the uterine epithelium. **B:** Luminal lipid droplets associated with the uterine epithelium. **C:** Detail of the contact interface between colloid of the carrier

matrix and the apical uterine epithelium. Bb, basal bodies; Ci, cilia; Co, colloid of the carrier matrix; De, desmosome; Ic, intercellular canaliculus; Mf, microfilaments; Mi, mitochondria; Mpt, middle piece of the tail; Mv, microvilli; Nu, nucleus of an epithelial cell; Pm, plasma membrane of the luminal border of an epithelial cell; Sv, secretory vacuoles.

not describe special sperm storage glands, and implied storage simply in the oviducal lumen (Rahn, '40; Ludwig and Rahn, '43). The presence of specialized sperm storage glands in the oviducts of snakes was initially described in viviparous (by lecithotrophy) females of *Thamnophis sirtalis* and *T. elegans* from San Francisco Bay (Fox, '56). Fox ('56) found a short, thick, convoluted area between the uterus and infundibulum in which "branched" (compound) alveolar glands contain sperm. SSTs in these *Thamnophis* were found to differ histologically from simple tubular glands of the uterus, and the infundibulum and vagina lack tubular glands (Fox, '56). Fox and Dessauer ('62) studied the oviduct in oviparous *Typhlops* and *Leptotyphlops*, in which females possess only the right oviduct. They again found simple, branched alveolar glands in the region between the infundibulum and uterus, but no sperm were present in the putative "SSTs" of any of the specimens examined.

Hoffman and Wimsatt ('72) conducted the only previous TEM observations on sperm storage in female snakes, and they also conducted extensive histochemical tests, using *Thamnophis sirtalis* from Ithaca, New York. They stated that SSTs are located on lateral and medial walls of the posterior infundibulum and consist of gland-like "crypts" that extend to the base of the mucosa. Typically, several sperm receptacles were found to communicate with the oviducal lumen via a common duct; thus, Hoffman and Wimsatt ('72) also characterized SSTs as compound alveolar glands. The necks of these SSTs, like the oviducal lining, are ciliated, but the basal cells of the crypts are unciliated and strictly secretory. Sperm heads often indent the apical cytoplasm of SSTs, and the authors suggested a "Sertoli cell-like" relationship, although some sperm resorption occurs (Hoffman and Wimsatt, '72).

Halpert et al. ('82) used light microscopy to study the annual cycle of sperm storage in female *Thamnophis sirtalis* collected in Manitoba prior to and after hibernation. They reported occurrence of fall and spring matings and storage of sperm in "tube-like" infundibular SSTs as well as vaginal "furrows." After fall mating, sperm are initially stored in furrows at the anterior end of the vagina. A PAS+ material associated with sloughed epithelium from the furrows associates with the sperm and serves as a "carrier matrix" to transport sperm to the infun-

dibular SSTs. In our results, we raised the possibility that the carrier matrix contains contributions from male secretory products as well. After spring mating, sperm in the SSTs from the fall mating are evacuated and degenerate, and sperm from the recent mating with new carrier matrices migrate to the SST area. Halpert et al. ('82) did not speculate whether, in the absence of spring mating, that the sperm stored from fall matings remain viable and are sufficient to fertilize eggs during ovulation in late June and early July.

Aldridge ('92) studied the annual oviducal cycle of oviparous *Tantilla coronata* using light microscopy. This species has a vestigial left oviduct into which eggs are not ovulated, but all the oviducal regions are present, including SSTs that store sperm. All oviducal glands were characterized as compound alveolar. As reported by Halpert et al. ('82) in *Thamnophis sirtalis*, sperm from matings in the late summer and fall overwinter in the anterior vagina (considered posterior uterus by Aldridge) and migrate into the SSTs in spring coinciding with vitellogenesis. The presence of sperm is not correlated with glandular hypertrophy, leading Aldridge to conclude that the glands are for mechanical protection of sperm rather than for nutrition.

Perkins and Palmer ('96) studied the oviduct of oviparous *Diadophis punctatus* using light microscopy and SEM. During the quiescent period, sperm are found in the "crypt-like folds" and lumen of the anterior vagina. In their Figure 6, these crypts are labeled "sperm receptacles" and appear to be tubular exocrine glands (Perkins and Palmer, '96). Sperm arrive in SSTs of the "uterine tube" (posterior infundibulum) during mid- to late vitellogenesis. The SSTs are described as having many branched, ciliated ducts leading to alveoli, so, once again, they comprise compound tubulo-alveolar glands. During gravidity, the SSTs become depleted of sperm.

Sperm storage glands

Contrary to previous studies, we did not find that oviducal glands containing sperm in *Seminatrix pygaea* are "alveolar." The glands in the SST area and anterior vagina containing sperm include both simple and compound tubular glands, with identical cytology. Cells of the distal portions of the oviducal evaginations that form the glands did not differ in size or cytology from cells

forming the more proximal portions of the ducts or from the adjacent oviducal lining. Ciliated cells are not limited to the orifice but occur intermixed with secretory cells in all portions of the glands.

In the SSTs of *Thamnophis sirtalis*, Hoffman and Wimsatt ('72) reported granules of sulfated mucopolysaccharides (apparently lacking in other oviducal glands), cytoplasmic glycogen, and lipid droplets. We found lipid droplets in the epithelium throughout the oviduct, but we have not observed glycogen. Our "secretory granules" in the SSTs stain positively with PAS and brilliant indocyanine 6B for proteins and weakly (or not at all) with Alcian blue 8GX at pH 2.5, which indicates carboxylated mucopolysaccharides, but not necessarily sulfated ones (Kiernan, '90). We suggest some mucoprotein is present, but the secretions probably are more complex.

We agree with Aldridge ('92) that the infundibular SSTs are for mechanical protection of the sperm rather than nutrition. Sperm not contained in SSTs most likely would be swept down the uterus by the first ovulated egg.

As noted by other workers (Hoffman and Wimsatt, '72; Blackburn, '98), the SSTs are not bordered by contractile elements (myo-epithelium or smooth muscle). Fox ('56) suggested that sperm are released from the SSTs simply by the pressure that the passing egg exerts on the oviducal lining.

Sperm migration

Sperm move anteriorly in the oviduct due to their own swimming ability, smooth muscle contractions, and/or the aid of cilia, which occur on some cells throughout the oviduct (Fox, '56; Blackburn, '98; Olsson and Madsen, '98). No chemical attraction seems involved, since the secretions of the lining and the glands are similar in different regions of the oviduct. Sperm may enter the orifices of glands simply as a continuation of a thigmotactic response, as suggested for sperm migration into salamander sperm storage glands by Hardy and Dent ('86). The first glands encountered are in the anterior vagina and posterior uterus, but any sperm entering these glands would be expelled during formation of the carrier matrix.

Halpert et al. ('82) stated that formation of the carrier matrix in *Thamnophis sirtalis* occurs by sloughing of the PAS+ epithelial border of the anterior vagina. Such a process seems consistent with our observations of

desquamated epithelial cells in the matrix, but we suggest that some of the unattached cells in the matrix could be phagocytes. Also, we propose a contribution to the carrier matrix from male secretory products based on the large volume of matrix relative to limited oviducal secretory activity, and differences in density between oviducal secretory vacuoles and the colloid of the matrix.

Halpert et al. ('82) found matrix formation in the anterior vagina, but we found an oviducal contribution to the matrix (Fig. 9) only in a region we consider posterior uterus. All glands in the uterus of *Seminatrix pygaea* are simple tubular, whereas the anterior vagina has compound tubular glands, so these areas are easily distinguished. We noted some desquamated or unattached cells in the vagina, but no matrix was observed around sperm in that area.

Halpert et al. ('82) found carrier matrix formation 24 h after spring mating. Some 48 h after mating, sperm, often associated with carrier matrix, appear in the SST region (Halpert et al., '82). The carrier matrix apparently dissipates after reaching the infundibulum (Halpert et al., '82). The role of the carrier matrix in sperm migration is unclear. Perhaps it protects the sperm and/or occludes orifices of more anterior uterine glands so the sperm bypass them. More anterior uterine glands, however, are rather inactive prior to ovulation and often have constricted orifices (in contrast to SSTs) or are filled with their own secretory products (e.g., Fig. 2D).

Sperm competition

Fox ('56) stated that the most posterior SSTs fill with sperm first, implying that sperm enter the first tubules encountered in which the orifices are not blocked by other sperm. Thus, in the event of multiple matings, the most anterior SSTs would contain sperm from the most recent mating. Since an ovulated egg would first encounter sperm from the most anterior SSTs, a last male paternity would appear to be favored, especially in small litters. Virtually all the literature on male mating success in reptiles, however, has reported a first male advantage results from multiple matings (Olsson and Madsen, '98). Olsson and Madsen ('98) noted that these studies may be confounded by a "timing effect"; experiments designed to determine male precedence in fertilization may not allow adequate time for sperm transport to the infundibulum in all participating

males. Also, Olsson et al. ('96) found that under conditions of multiple matings of the lizard *Lacerta agilis*, more distantly related males sired a larger percentage of the offspring, implying females (or eggs) can discriminate between sperm. Obviously, much more work combining morphological, ecological, and genetic data is necessary before we can elucidate the relationship between sperm storage and sperm competition in any reptile.

Reproductive cycle

Our results agree with Seigel et al. ('95) that rapid follicle growth occurs after hibernation, ovulation occurs in early June, and parturition occurs in early August. We differ in our data on reproductive frequency. Over 4 years, Seigel et al. ('95) found 77.3–91.7% of females gravid, indicating an annual reproductive cycle in most females. In our sample, which is much smaller but collected using the same techniques, we found some evidence for at least a biennial reproductive cycle for females. Two of the four females collected and sacrificed in May prior to the period of ovulation (June) were nonvitellogenic and thus were not reproducing in that year (Table 1). Also, one of the four specimens from the June sample (ovulatory period), and three of four sacrificed 30 July (period of gravidity) were not reproducing. One cannot be certain that all five specimens examined from October had skipped the last breeding season, but their oviducts were not distended, as one may expect after housing fetuses (Blackburn, '98).

No evidence for a fall mating was found. Five females collected 29 September–2 October, about a month before the first <0°C temperatures (which occurred 7 November and probably limited further surface activity for these snakes) lacked sperm anywhere in their oviducts.

Finally, the occurrence of sperm in the anterior vagina and posterior uterus of a nonvitellogenic female collected in June is curious. Sperm in a carrier matrix indicate a recent mating, but this snake was not ovulating eggs in the current breeding season. We are left to wonder whether this female was exhibiting the potential to store sperm for a year (i.e., the next opportunity to ovulate) or whether this phenomenon indicates a decoupling of mating behavior from oogenesis.

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