Female Sperm Storage in Reptiles

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ABSTRACT Internal fertilization and oviparity most likely are sympleiomorphies for modern reptiles, and viviparity has evolved independently numerous times in Sauria and Serpentes. Oviducal sperm storage is known in females of all taxa except Amphibia. However, in Rhynchocephalia and Crocodilia, sperm storage is poorly studied, and specialized sperm storage tubules (Ssts) are unknown. We use the molecular phylogenetic hypothesis [(Chelonia+Archosauria) (Squamata)] to trace evolution of sperm storage characters. Ssts arose independently in Chelonia and Squamata. Turtles possess albumen-secreting glands in the anterior half of the oviduct (the tuba or isthmus), and the most distal of these glands also serve as Ssts; in addition, some turtles possess Ssts in the adjacent segment of the oviduct, the uterus. Squamates lack albumen-secreting glands, and the ancestral state is possession of Ssts in the posterior infundibulum (uterine tube). Secondarily, iguanids have evolved vaginal Ssts. In this paper, we present the first ultrastructural observations on vaginal Ssts in lizards, using Anolis sagrei (Polychrotidae). Proximally, the neck of these simple tubular glands continues the alternation of ciliated and secretory cells lining the lumen of the vagina. However, the epithelial cells of the distal sperm storage area are neither secretory nor ciliated. The Ssts of Anolis are more similar to those of birds more than to infundibular receptacles in snakes and lizards. J. Exp. Zool. 292:187–199, 2002. © 2002 Wiley-Liss, Inc.

Female, as well as male, sperm storage is an obligatory part of the reproductive cycle in many reptiles. Sperm production, mating, and ovulation are out of phase with one another in numerous species (Fox, '52; Saint Girons, '82; Schuett, '92). Thus, storage by either males or females promotes the wide dissemination, over time, of gametes produced in a narrow time window (Gist and Jones, '87). The selective advantage of sperm storage in reptiles does not involve long-term production of multiple clutches from a single mating, but rather the facilitation of female choice and sperm competition (Olsson and Madsen, '98).

The role of sperm storage in behavioral and ecological aspects of the reproductive biology of reptiles has been reviewed extensively (Saint Girons, '73; Devine, '84; Schuett, '92; Olsson and Madsen, '98), and this paper is not concerned with these subjects. This paper also is not directly concerned with the physiology of sperm storage; our knowledge about how viability is maintained and capactiation is achieved is quite limited (Hoffman and Wimsatt, '72; Depeige, et al., 87; Averal et al., '92; Manimekalai and Akbarsha, 92). Rather, herein we consider the anatomy and phylogeny of sperm storage in reptiles and extend observations made by Gist and Jones ('87) in the last review on these topics.

We actually know very little about the anatomy of sperm storage in reptiles. Ultrastructural studies, especially using transmission electron microscopy (TEM), are essential to study sperm/epithelial interactions during sperm storage. Yet, out of 5,400+ species of reptiles, we have TEM observations on female sperm storage tubules (Ssts) for just one turtle (Gist and Fischer, '93), one lizard (Bou-Resli et al., '81), and two snakes (Hoffman and Wimsatt, '72; Sever and Ryan, '99). We are unaware of any ultrastructural study devoted to male sperm storage in the reptilian epididymis or vas deferens. A number of papers, however, deal at least peripherally with the cytology of male sperm storage in reptiles (Weil, '84; Esponda and Bedford, 87; Carcupino et al., '89; Gist et al., '92; Newton and Trauth, '92). A review of male sperm storage in reptiles, however, seems premature, so this paper is limited to female sperm storage in the oviduct.

The purpose of this paper is three-fold. First, we consider current views on evolutionary relationships among reptiles and propose general hy-
potheses concerning the phylogeny of female sperm storage among reptilian taxa. Second, we undertake a more detailed review of the anatomy of female Ssts in various reptilian groups in which these structures have been studied and the evidence for sperm storage in other taxa in which Ssts are currently unknown. Finally, we present the first TEM observations on vaginal Ssts in reptiles using *Anolis sagrei* (Polychrotidae).

**MATERIALS AND METHODS**

McClade 3.0 (Maddison and Maddison, ’92) was used to map sperm storage characters. Phylogenies were adapted from Pough et al. (’98), Hedges and Poling (’99), and Kumazawa and Nishida (’99).

Specimens of *Anolis sagrei* were collected by hand 26–29 June 1999 and 12–15 July 2000, at Long Key, Florida. After capture, the lizards were euthanized by cervical transection, and reproductive tracts were removed and fixed in formaldehyde/glutaraldehyde, 2.5% each in 0.1 M sodium cacodylate buffer, pH 7.4. After storage, the tissue from eight females was prepared for embedding in glycol methacrylate (JB-4, Polysciences) for light microscopy or embedding in epoxy resin (Epon 812, Electron Microscopy Sciences) for TEM.

For light microscopy, tissue was rinsed in DDOH and dehydrated in a graded series of ethanol before embedding in JB4. Sections (2 μm thick) were stained with methylene blue-basic fuchsin. For TEM, tissues were rinsed in sodium cacodylate buffer, post-fixed in 2% osmium tetroxide in cacodylate buffer, dehydrated in ethanol, and cleared in propylene oxide. Sections (700 nm) were placed on uncoated copper grids, stained with uranyl acetate and lead citrate. Histological and cytological methods followed Dawes (‘79) and Kiernan (‘90).

**RESULTS AND DISCUSSION**

**Phylogenetic considerations**

The Class Reptilia contains includes Crocodilia (22 species), Chelonia (turtles, 260 species), Rhynchocephalia (two species), Amphisbaenia (135 species), Sauria (lizards, 3,200 species), and Serpentes (snakes, 1,800 species; Pough et al., ’98). Amphisbaenids, lizards, and snakes comprise the Squamata; and rhynchocephalians are grouped with squamates to form the Lepidosauria. Crocodilians are grouped with birds in the Archosauromorpha, traditionally considered the sister taxon to Lepidosauria, and Chelonia usually is deemed the sister taxon to Archosauromorpha+Lepidosauria (Fig. 1; Pough et al., ’98). Recent molecular work, however, indicates that Chelonia and Archosauromorpha are closest sister-taxa, and thus Chelonia+Crocodilia is the sister group of Squamata (Fig. 1; Hedges

![Diagram](image-url)

Fig. 1. Recent molecular studies have changed the way we traditionally view the phyletic relationships among amniotes. We adopt the view that turtles form sister-group relationships with crocodilians and birds. Because we have no data on the presence of Ssts in crocodilians, we omit them from the cladogram that we use to trace reproductive characters.
and Poling, '99; Kumazawa and Nishida, '99). Rhynchocephalians may have their closest sister-group relationships with Chelonia+Archosauria, rather than Squamata (Hedges and Poling, '99), thus casting doubt on the Lepidosauria as a natural group.

Character states were determined for location of sperm storage tubules and various other reproductive characters for reptiles, birds, and mammals (Tables 1, 2). Birds are well-known to possess Ssts at the uterovaginal junction (Bakst, '87), and mammals either lack Ssts, or store sperm in the uterus or uterotubal (isthmus) regions (Racey, '79; Bedford and Breed, '94; Suarez, '98). Mapping of these characters on the phylogeny chosen for this study reveals that an ancestral state for Ssts in amniotes cannot be determined and that Ssts evolved independently in birds, turtles, squamates, and mammals (Fig. 2).

The ancestral state for squamates is possession of infundibular Ssts. The lizard data from Table 1 can be mapped on a phylogeny of lizards taken from Pough et al. (98). The result (Fig. 3) indicates that vaginal Ssts are a specialization found in the Iguania, and that some iguanids have also either retained infundibular Ssts or re-evolved them.

**Variation in female sperm storage**

**Crocodilia**

Davenport ('95) reported that a female caiman (Paleosuchus palpebrrosus) laid 16 eggs, in at least one of which an embryo developed, 488 days after isolation from a male. Ferguson ('85) failed to find sperm storage structures in the alligator (Alligator mississippiensis) or the crocodile (Crocodylus niloticus). Palmer and Guillette ('92) reported that the oviduct of A. mississippiensis has separate uterine regions for formation of the eggshell membranes and the calcareous layer, similar to those of birds and unlike other reptiles.

**Rhynchocephalia**

Dawbin ('62) stated that 10 months may elapse between copulation and oviposition in the tuatara (Sphenodon punctatus). No sperm storage structures, however, have been found in the oviduct (Gabe and Saint Girons, '64).

**Amphisbaenia**

To our knowledge, no histological descriptions exist on the oviduct of any amphisbaenid, and we know of no observations on the potential for sperm storage. However, because of the close sister-group relationship with Sauria (Pough et al., '98), we believe that oviducal Ssts will be found in amphisbaenids.

**Chelonia**

The first description of Ssts in turtles was in the box turtle (Terrapene carolina) by Hattan and Gist ('75). Subsequently, Ssts were reported from 12 species representing six families (Gist and Jones, '89), and such structures probably exist in the remaining seven families. Turtles have an extensive area in the anterior half of the oviduct, called the tuba by Gist and Jones ('87), in which albumen-secreting glands occur. In the most caudal end of this region, some albumen glands also serve as Ssts. In two species, Sternotherus odoratus and Gopherus polyphemus, Ssts have also been reported from the uterine region of the oviduct near the uterovaginal junction (Palmer and Guillette, '88; Gist and Congdon, '98). Whether the storage tubules are in the tuba or in the uterus, sperm are found in glands located at the periphery and not the center of the major glandular regions (Gist and Congdon, '98).

The only ultrastructural observations on the Ssts of turtles are TEM studies on T. carolina by Gist and Fischer ('93). They reported that the albumen glands containing sperm are not differ cy-

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**TABLE 1. Literature on location of sperm storage tubules (Ssts) in lizards**

<table>
<thead>
<tr>
<th>Family</th>
<th>D¹ infundibulum</th>
<th>D² vagina</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agamidae</td>
<td>X</td>
<td>X</td>
<td>Saint Girons, '73; Kumari et al. '90</td>
</tr>
<tr>
<td>Anguidae</td>
<td>X</td>
<td></td>
<td>Saint Girons, '73</td>
</tr>
<tr>
<td>Chameleonidae</td>
<td></td>
<td>X</td>
<td>Saint Girons, '82</td>
</tr>
<tr>
<td>Eublepharidae</td>
<td>X</td>
<td></td>
<td>Cuellar, '66</td>
</tr>
<tr>
<td>Gekkonidae</td>
<td>X</td>
<td></td>
<td>Cuellar, '66; Bou-Resli et al., '81; Murphy-Walker and Haley, '96; Girling et al., '97</td>
</tr>
<tr>
<td>Iguanidae</td>
<td>X</td>
<td>X</td>
<td>Cuellar, '66; Adams and Cooper, '88</td>
</tr>
<tr>
<td>Polychrotidae</td>
<td></td>
<td>X</td>
<td>Fox, '62; Conner and Crews, '80</td>
</tr>
<tr>
<td>Sphenidae</td>
<td></td>
<td>X</td>
<td>Saint Girons, '62; Schaefer and Roeding, '73</td>
</tr>
</tbody>
</table>
TABLE 2. Character states and their polarities for sperm storage tubules (Ssts) and some other reproductive characters in reptiles

<table>
<thead>
<tr>
<th>A - Fertilization</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - external</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>1 - internal</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B - Ovary</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 - solid</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 - hollow</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C - Albumen</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 - present</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 - absent</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D - Ssts</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 - absent</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 - posterior infundibulum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 - tubal albumen glands</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 - uterovaginal</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 - vaginal</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>5 - uterus or uterotubal</td>
<td></td>
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</tbody>
</table>

*Uterine glands also reported as Ssts in two species of turtles.

Fig. 2. Ssts evolved independently in birds, turtles, squamates, and mammals. Among squamates, the ancestral state is infundibular Ssts, and vaginal Ssts have subsequently evolved in some lizards.

Serpentes

The first observations on sperm storage in the oviduct of a female snake were made by Rahn ('40), but specialized Ssts were not described until Fox ('56). No Ssts were found in the brown tree snake, Boiga irregularis by Bull et al. (’97), but these glands are no doubt widespread in snakes, and numerous accounts, often anecdotal, exist on long-term sperm storage in various species (Sever and Ryan, '99). Some reported incidences of long-term sperm storage in snakes, however, may actually represent cases of facultative parthenogenesis (Schuett et al., ’97). The only ultrastructural studies on the Ssts of snakes concern TEM of the garter snake, Thamnophis sirtalis (Hoffman and Wimsatt, ’72); scanning electron microscopy (SEM) of the ringneck snake, Dipsosaurus punctatus (Perkins and Palmer, ’96); and both TEM and SEM of the black swamp snake, Seminatrix pygaea (Sever and Ryan, ’99).

Ssts occur in a short region between the infundibulum and the uterus called the uterine tube (Perkins and Palmer, ’96) or simply, the posterior infundibulum, to distinguish this region from the more anterior, aglandular infundibulum (Fox, ’56; Blackburn, ’98; Sever and Ryan, ’99). In many snakes in temperate regions, however, mating occurs in fall, and the sperm are held over winter in vaginal or uterine sperm receptacles and migrate to the tubal Ssts for a brief period of storage prior to ovulation in the spring (Halpert et al., ’82; Aldridge, ’92; Perkins and Palmer, ’96).

In their TEM study on infundibular Ssts in the garter snake, Hoffman and Wimsatt (’72) characterized the Ssts as compound alveolar glands. The necks of the Ssts, like the surrounding oviducal lining, contain ciliated cells, but the distal bulbs...
are not ciliated and strictly secretory. Histological tests found evidence of sulfated mucopolysaccharides, glycogen, and lipids. Sperm nuclei often indent the apical cytoplasm of Ssts, leading the authors to suggest a “Sertoli-cell-like” relationship, although they also observed sperm resorption (Hoffman and Wimsatt, '72).

Sever and Ryan (‘99) reported that Ssts in the infundibulum and vagina of *Semimatrix pygaea* are simple or compound tubular (Fig. 4A). In both areas, ciliated and secretory cells line the entire tubule, so the glands do not differ significantly from the adjacent oviducal lining (Fig. 4B–C). The vaginal glands (Fig. 5A–B) are similar to infundibular Ssts except that the sperm are aligned more orderly, vaginal nuclei are more euchromatic, and the secretory vacuoles contain more flocculent material. Also, infundibular Ssts react positively with periodic acid-Schiff reagent (PAS) for neutral carbohydrates; whereas vaginal Ssts, in addition, stain positively with alcin blue at pH 2.5 for carboxylated glycosaminoglycans. Unlike *Thamnophis sirtalis*, cytoplasmic glycogen does not occur. Sever and Ryan (‘99) also reported positive histochemical tests for proteins and lipids. Sperm occasionally are found embedded in the apical cytoplasm of the oviducal lining of a Sst, but Sever and Ryan (‘99) reported no evidence of sperm degeneration, support, or nutrition.

Sever and Ryan (‘99) also described the ultrastructure of an extraordinary luminal matrix containing sperm in the posterior uterus (Fig. 5C–D). This “carrier matrix” was originally described in the posterior oviduct of recently mated *Thamnophis sirtalis* by Halpert et al. (‘82). The matrix consists primarily of a dense colloid of secretory material but also contains desquamated epithelial cells and membranous structures (Fig. 5C–D). At least some of the matrix is secreted by the epithelial lining and uterine glands. Secretions from the uterine epithelium, however, do not seem sufficient to account for the magnitude of the matrix (Sever and Ryan, ‘99). The matrix, therefore, may also include contributions from male secretory products. Halpert et al. (‘82) proposed that the carrier matrix facilitates the transport of sperm to the infundibular Ssts.

As reported for turtles, the glands used for sperm storage in snakes do not appear specialized for this function. We agree with Aldridge (‘92) and Sever and Ryan (‘99) that the infundibular Ssts are for mechanical protection of the sperm rather than for nutrition. Sperm not contained in Ssts most likely would be swept away by the first ovulated egg.

**Sauria**

Ssts were first described in the oviducts of the chameleons, *Chamaeleo basiliscus*, *C. chamaeleon*, and *C. lateralis* (Saint Girons, ‘62) and the anolis, *Anolis carolinensis* (Fox, ‘63). Other literature concerning histological studies is given in Table 1. The only ultrastructural studies are SEM of the SST area in *A. carolinensis* (Conner and Crews, ‘80) and several species of geckos (Girling et al., ‘97, ‘98) and a TEM study on Ssts of the gecko *Acanthodactylus scutellatus* (Bou-Resli et al., ‘81). Both vaginal and infundibular (tubal) Ssts are known (Table 1), and some species in the Iguanidae (Cuellar, ‘66) and Agamidae (Saint Girons, ‘73) apparently have Ssts in both areas.

Bou-Resli et al. (‘81) described the infundibular Ssts of *Acanthodactylus scutellatus* females that were isolated from males and sacrificed over a period of four months. The Ssts are simple tubular glands whose openings into the oviduct are nearly completely obscured by cilia. Cilia decrease in number distally in the Ssts, although even at the “blind end of the tubule” a few cilia remain. Secretory cells contain oval, electron-dense vacuoles that occupy most of the apical area of the cell. Rod-shaped mitochondria and free ribosomes are numerous in the cytoplasm, but rough endoplasmic reticulum, lysosomal bodies, and Golgi complexes occur rarely.

Bou-Resli et al. (‘81) found head and tail regions of sperm embedded in the cytoplasm of the Ssts or in intercellular spaces. Cytoplasmic membranes are intact around the invaginated sperm. The authors suggest that secretions of the Ssts have a nourishing function for sperm, but they also proposed that the Ssts provide protection for sperm (Bou-Resli et al., ‘81).

**Histology and ultrastructure of the vaginal Ssts of Anolis sagrei**

Fox (‘63) was the first to report sperm in vaginal Ssts of a lizard, in *Anolis carolinensis*, which is closely related to *A. sagrei*. Fox reported that the oviduct consists of three parts. The proximal infundibulum is thin-walled and lined by ciliated and nonciliated columnar epithelium that is apparently aglandular. The middle uterus contains coiled tubular shell glands. The vagina consists of a thin, anterior vaginal tube and a thick, posterior vaginal pouch. From the bottom of longitudinal grooves in the anterior two-thirds of the vaginal tube, Fox (‘63) found small tubules, Ssts, extending deep in the thick tunica propria and passing more or less parallel to the folds. In many
Fig. 4. Infundibular Ssts in the black swamp snake, *Seminatrix pygaca* after Sever and Ryan ('99). (A) is a light micrograph, and (B–D) are electron-micrographs. A: Sagittal section through the Sst area in the infundibulum. B: Sperm in the oviducal lumen of the posterior infundibulum. C: Proxi-
Fig. 5. Transmission electron micrographs of vaginal sperm storage glands and sperm in the uterus of the black swamp snake, *Seminatrix pygaea* after Sever and Ryan (99). A: Distal portion of a vaginal gland containing sperm. B: Proximal end of a vaginal gland containing sperm. C: Sperm in the carrier matrix in the uterus. D: Detail of the carrier matrix.

Ci = cilia; Co = colloidal matrix; Ds = desquamated epithelium; Ld = lipid droplet; Lu = lumen; Mi = mitochondria; Mpt = middle piece of the tail; Ms = membranous structures; Nu = nucleus; Nuds = nucleus in a desquamated cell; Or = orifice; Sn = sperm nucleus; Sp = sperm; Sv = secretory vacuole.
of these tubules, depending on the time elapsed since the last insemination, are bundles of sperm. The ciliated cells associated with the rest of the lining do not extend into the distal areas of the Ssts where sperm are stored.

Conner and Crews ('80) refined the description of the vagina of Anolis carolinensis provided by Fox ('63). They noted that the anterior vaginal tube of Fox ('63) consists of two portions, the utero-vaginal transition and an area just posterior to the transition, which consists of a thin tube flanked on both sides by openings that extend anteriorly and end blindly in the oviducal wall. The entire anterior half of the vagina is highly folded with ciliated and non-ciliated cells alternating in the epithelium. The Ssts are located in the utero-vaginal transition.

Whether one refers to the Sst area in Anolis as the anterior vaginal tube (Fox, '63) or the utero-vaginal transition area (Conner and Crews, '80) could be significant as further comparative work emanates. In our specimens of A. sagrei, Ssts are clearly limited to the region immediately adjacent to the uterus where eggs develop prior to oviposition (Fig. 6A). This is equivalent to the region where Ssts are found in birds, and Bakst ('87) and others have adopted the term utero-vaginal junction to describe the location of such glands. We are unclear whether Ssts are limited to this utero-vaginal junctional area in other lizards in which vaginal Ssts are known (Cuellar, '66; Adams and Cooper, '88; Kumari et al., '90). Thus, to be as precise as possible, we refer to the Sst region in A. sagrei as the utero-vaginal junctional area; we do not use the term "transition," because this region is unique by virtual of the Ssts and does not represent a gradation between the uterus and the remainder of the vagina.

Many species of Anolis are known to lay single eggs every 10–14 days during a two- or three-month breeding period, and the ovaries and oviducts alternate in this process (Conner and Crews, '80). This situation apparently exists in A. sagrei from the Florida Keys, and all the females that were dissected from our April samples contain one well-developed egg in one uterus, as well as a more recently ovulated egg in the other uterus (Fig. 6A). The egg is shelled by the time it reaches the vagina, so sperm apparently migrate up the oviduct to the infundibulum to fertilize eggs; sperm have been found in the infundibulum 6–24 hrs after mating (Conner and Crews, '80). Sperm stored in the Ssts may be used to fertilize successive eggs over several months. The tubules thus provide protection for sperm during passage of eggs through the vagina (Conner and Crews, '80).

As reported by Fox ('63) and Conner and Crews ('80), the lining of the oviduct in the utero-vaginal junction area consists of ciliated cells and non-ciliated secretory cells, a typical situation in vertebrate oviducts (Fig. 6B). This lining extends into the neck portion of the Ssts (Figs. 6C, 7A–C). We found some variation among females in extent of secretory activity in the Ssts and adjacent oviducal lining, and this variation seems related to the amount of sperm present. Secretory vacuoles are larger, and perhaps contain a different product, in specimens in which the Ssts contain more sperm (compare Figs. 6C and 7A).

The distal portion of the Ssts, however, contain cells that lack secretory vacuoles and cilia (Figs. 6D, 7B–D). The distal ends contain the most sperm and are presumably the primary storage area (Fox, '63). Sperm may be clustered in bundles (Fig. 6C) or align themselves more randomly (Fig. 6D), perhaps depending on the numerical density of the sperm.

No contractile elements are associated with the basal lamina of the Ssts. Collagen fibers occur superficial to the Ssts (Fig. 7B,D) and the muscularis is thin (Fig. 6D). The role, if any, of the tissues surrounding the Ssts in sperm transport into and out of the Ssts remains unresolved (Conner and Crews, '80; Bou-Resli et al., '81).

The epithelium of the distal portions lacks synthetic organelles and, indeed, appears almost syncytial (Fig. 8A,B). The intercellular canaliculi are marked by thickened, felt-like areas (Fig. 8A), which, on closer examination, reveal a lack of continuity of the plasma membranes between desmosomal areas (Fig. 8B). Scattered oval, dense mitochondria, and a few clear vacuoles are the only cytoplasmic structures noted in most cells (Fig. 8B).

Finally, parts of a few sperm are embedded in secretory cells of the neck region (Fig. 8C,D). These parts include portions of the tail, as well as sperm nuclei. Embedded sperm are found among secretory vacuoles in felt-like material and simply in the cytoplasm. Microfilaments occur occasionally close to the embedded sperm, but the sperm do not seem to be enclosed in membrane bound vacuoles (Fig. 8D). No sperm are embedded in ciliated cells or in the distal nonsecretory cells. The significance of embedded sperm (phagocytosis? nutrition?) needs further investigation.
Fig. 6. *Anolis sagrei* collected in June 1999 (A) is a dissection of the reproductive tract, and (B–D) are light micrographs. B: Sagittal section through the oviduct at the uterovaginal junction. C: An Sst showing the proximal secretory and ciliated neck portion (Np) and the distal portion (Dp) that lacks secretory and ciliated cells but serves as the primary sperm storage area. Note crowded cluster of sperm aligned together along their long axes. D: Distal portion of an Sst, illustrating random arrangement of sperm. Cl = cilia; Cl = cloaca; Dp = distal portion of an Sst; Inf = infundibulum; Ms = muscularis; Nu = nucleus; Np = neck portion of an Sst; Splu = sperm in the lumen; Sst = sperm storage tubule; Ut = uterus; Vg = vagina.
Fig. 7. *Anolis sagrei* collected June 1999 (A) is a light micrograph and (B–D) are transmission electron micrographs. A: Neck portion containing secretory and ciliated cells and distal portion containing sperm. B: Same as A, showing ultrastructure. C: Sperm in the neck portion of an Sst. D: Sperm in the distal portion of an Sst. Bb = basal bodies; Cf = collagen fibers; Ci = cilia; Fm = felt-like material; Lu = lumen; Mi = mitochondria; Nu = nuclei; Sm = secretory material; Sp = sperm; Splu = sperm in the lumen; Sat = sperm storage tubules; Sv = secretory vacuoles; Va = vacuole; Vp = visceral pleuroperitoneum.
Fig 8. *Anolis sagrei*, transmission electron micrographs through the Ssts of the same specimen as used for Fig. 7. A: Overview of the epithelium of the distal portion of an Sst storing sperm. B: Detail of the cytoplasm of the distal portion of an Sst; note the lack of organelles involved in synthetic processes and the almost syncytial appearance of the cytoplasm. C: Sperm in the lumen and embedded in the apical cytoplasm of a neck cell of a Sst. D: Detail of sperm in the cytoplasm of a neck cell. Bl = basal lamina; Cf = collagen fibers; De = desmosomes; Fm = felt-like material; Lu = lumen; Mf = microfilaments; Mi = mitochondria; Mpt = middle piece of the tail; Nu = nucleus; Ppt = principle piece of the tail; Sm = sperm nucleus; Sp = sperm; Sv = secretory vacuole; Va = vacuole.
TABLE 3. Sst characteristics based on TEM studies

<table>
<thead>
<tr>
<th>Species</th>
<th>Location</th>
<th>Type</th>
<th>Linings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seminatrix pygaea</td>
<td>Infundibulum, vagina</td>
<td>Simple tubular</td>
<td>Ciliated and secretory cells throughout</td>
</tr>
<tr>
<td>Thamnophis sirtalis</td>
<td>Infundibulum</td>
<td>Compound tubulooalveolar</td>
<td>Ciliated proximally secretory distally</td>
</tr>
<tr>
<td>Acanthodactylus scutellatus</td>
<td>Infundibulum</td>
<td>Simple tubular</td>
<td>Ciliated and secretory cells throughout</td>
</tr>
<tr>
<td>Anolis sagrei</td>
<td>Uterovaginal junction</td>
<td>Simple tubular</td>
<td>Ciliated and secretory cells proximally</td>
</tr>
<tr>
<td>Domestic fowl</td>
<td>Uterovaginal junction</td>
<td>Simple tubular</td>
<td>Ciliated and secretory cells proximally</td>
</tr>
</tbody>
</table>

CONCLUSION

Table 3 presents a summary of some characteristics of squamate Ssts based on the four TEM studies available. The infundibular Ssts of the snakes Thamnophis sirtalis (Hoffman and Wimsatt, '72) and Seminatrix pygaea (Sever and Ryan, '99) and the gecko Acanthodactylus scutellatus (Bou-Resli et al., '81) differ in details of tubular complexity and extent of ciliation that do not seem major and perhaps reflect, to some degree, the interpretation of the observer. These putative differences may assume more phylectic or functional importance as additional taxa are examined.

Our findings on Anolis sagrei, however, reveal one striking difference between the uterovaginal Ssts in this species and the infundibular Ssts in the other reptiles. Ciliated and secretory cells are limited to the neck portion of the Ssts in A. sagrei, and the distal portion, where sperm are concentrated, lacks cilia and secretory products. It is worth remarking that this situation is identical to the condition reported for uterovaginal Ssts in domestic fowl (Bakst, '87). In addition to the anatomy of their Ssts, A. sagrei and these birds share the traits of an extended period of continued egg production resulting from infrequent matings. The similarity of reproductive characters in an iguanid lizard and birds is perhaps another example of convergence due to similar functional adaptations and the design constraints of the vertebrate oviduct and sperm (Sever et al., 2001).

Future studies will determine whether similar convergence of form and function occurs with sperm storage adaptations among other reptiles and other vertebrates. As with other symplesiomorphic morphological characters, discerning subsequent specializations that are phylogenetically relevant from those that are homoplastic will be a challenge. Sperm storage, however, is such an intrinsic part of the reproductive cycle of reptiles that the phenomenon deserves attention in any study on reptilian natural history. Finally, histological studies are an excellent means of distinguishing incidences of sperm storage from occasions of facultative parthenogenesis. Parthenogenesis apparently is more widespread among reptiles than formerly believed and could account for some reports of exceptional long-term sperm storage (Schuett and Roeding, '97).

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LITERATURE CITED


REPTILE SPERM STORAGE


