

Sperm Transfer and Storage in the Lizard, *Anolis carolinensis*

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ABSTRACT The relationship of the hemipenis to the cloaca in copula and sperm storage and transport in the female oviduct were studied in *Anolis carolinensis* using light and scanning electron microscopy. During copulation, the hemipenis does not penetrate beyond the cloaca, but the two apical openings of the bifurcate sulcus spermaticus appose the openings of the oviducts from the cloaca. Sperm enter the sperm storage tubules between 2 and 6 hr after insemination and small amounts of sperm reach the infundibulum 6 to 24 hr following mating. Sperm storage tubules are embedded in the wall of the utero-vaginal transition, and are formed by the folding and fusion of the oviducal epithelium. The importance of the hemipenile-cloacal relationship and the role of sperm storage in the life history of *A. carolinensis* are discussed.

Male squamate reptiles have functionally separate, bilaterally symmetrical reproductive tracts complete with paired intromittent structures called hemipenes. Female squamates also have functionally separate, bilaterally symmetrical reproductive tracts; however, the female tract terminates in a single posterior cloaca. This system raises many important questions concerning the laterality of sperm transfer and fertilization. Investigation of the positional relationship of the male hemipenis to the female cloaca and oviducts in copula is a powerful approach to the resolution of these questions. In snakes, "copulatory adjustment" (hemipenile-cloacal relationship) has been studied by Pope ('41), Ludwig and Rahn ('43), Edgren ('53), and Inger and Marx ('62). These were observational studies, however, and no systematic study of this problem has been undertaken. Furthermore, no study of any kind has been conducted on the hemipenile-cloacal relationship in lizards.

In the lizard *Anolis carolinensis*, females exhibit a unique pattern of reproduction in which the two ovaries alternate in the production of single ova throughout the reproductive season (Hamlett, '52; Crews, '73a; Licht, '73). Male *A. carolinensis* intromit a single hemipenis during copulation, and tend to alternate in the use of the paired organs (Crews, '78; Figs. 1,2). Males also tend to intromit with the hemipenis ipsilateral to the ovary with the largest (preovulatory) follicle in the female (Crews, 1980).

Crews (1980) has speculated that the hemipenis could be intromitted into the ipsilateral oviduct, thus fertilizing the preovulatory follicle.

This study of the lizard, *Anolis carolinensis*, was undertaken with three goals in mind: 1) to elucidate the positional relationship of the male and female reproductive organs in copula to gain insight into the mechanism of sperm transfer; 2) to monitor sperm movement in the oviduct during the 48 hour period following insemination; and 3) to study the structure and function of the sperm storage tubules in the female reproductive tract.

MATERIALS AND METHODS

Animal maintenance

Male and female *Anolis carolinensis* were purchased from the Snake Farm, La Place, LA, during fall 1978. Males were housed in 6-mm wire mesh cages divided into twelve individual compartments, each measuring 24 × 8.5 × 30.5 cm; each compartment contained a water bottle, food dish, and stick for perching. Females were housed in 6-mm wire mesh group cages each measuring 46 × 49 × 30.5 cm and containing 15-20 lizards. Each group cage was equipped with water and food bowls; plants and twigs were provided for perches. Cages were kept in environmental chambers (Sherer-Gilet RI-48-BG-LLTP) providing a 14L:10D photic cycle with a corresponding 32°C:23°C daily thermal cycle and constant humidity of

70–80%. Animals were force-fed one *Tenebrio* larvae daily to insure adequate nutrition for rapid testicular and follicular growth; also, *Tenebrio* larvae, crickets, and water were available ad libitum.

Hormonal regimen

To insure high levels of testicular and ovarian activity, both males and females received a minimum of 14 daily injections of 10 μg NIH-FSH-S12 in 10 μl reptilian saline (Licht, '70); injections were subcutaneous and given one hour after onset of the light cycle.

Matings and dissection

Hormone-primed females (N = 18) were introduced into the cages of individual hormone-primed males and allowed to mate. Females were removed from the cages upon termination of mating and sacrificed by decapitation immediately or at intervals of 2, 4, 6, 18, 24, and 48 hr after mating. The oviducts were exposed by a ventral incision and the fat bodies and intestine were removed. The body was then severed transversely just anterior to the ovaries, and the lower half placed directly into fixative. This method prevented damage to the tissues and displacement of sperm which would result from handling of the oviduct during dissection; all oviducts were fixed within 15 min after decapitation.

Histology

Light microscopy

Following in situ fixation in Bouin's solution, the oviducts were removed from the body and inbedded in paraffin. Oviducts were sectioned longitudinally and transversely at seven and ten microns and stained with hematoxylin and eosin (Gray, '64).

Scanning electron microscopy

Two animals were dissected as above; the utero-vaginal transition of the oviduct (Fig. 5) was then removed and fixed in 10% buffered formalin for three days. Samples were then bisected longitudinally and returned to fixative for 24 hr. The tissues were washed in water, dehydrated in alcohol, critical-point dried in CO_2 , coated with gold-palladium, and viewed with an AMR 1000 scanning electron microscope.

In copula freezing

Two hormone-primed male and female pairs were allowed to mate in a wire mesh cylindrical cage measuring 6 cm high and 14 cm in diameter. Five minutes after intromission, the cage

was quickly immersed in 8 cm of dichlorodifluoromethane (Allied Chemical Genetron-12) in a foam ice bucket. This froze the animals in copula. The hemipenis was severed at the base, the female opened ventrally, and the entire cloaca removed, including the severed hemipenis. This tissue was cryostatically sectioned longitudinally and transversely at 40 μm , dried, fixed in 10% formalin, and stained with hematoxylin and eosin.

Sperm counts

Using two females sacrificed 48 hr after mating, five storage tubules on each side of the oviduct were selected randomly for sperm counts. Tubules were followed through serial sections (longitudinal, 7 μm) with the aid of tracings made with a camera lucida attachment on a Zeiss standard microscope. Three counts were made for each tubule in each section with a hand counter at 500 \times . The means of each set of three counts were summed to give an estimate of total number of sperm per tubule. Measurements of these tubules were also made with an eyepiece reticle to provide an estimate of tubule size; tubule length was estimated by multiplying the number of sections that the tubule appeared in by the section thickness (7 μm). Estimates of numbers of tubules per animal were also made using light microscopy.

RESULTS

Hemipenile morphology

For the description of hemipenile morphology, the standard nomenclature of Dowling and Savage ('60) will be used. Examination of each intact, everted hemipenis reveals a simple clavate structure with a bifurcate sulcus spermaticus. The ornamentation is differentiated, calculated apically, and flounced basally, with a disked apical structure (Figs. 3, 4). Studies of the longitudinal sections of the frozen hemipenis show the bifurcation of the sulcus and its distal openings in the depressions on either side of the apical surface (Fig. 6).

Oviducal morphology

The female reproductive tract of *A. carolinensis* consists of paired ovaries and oviducts which terminate in a single posterior cloaca. The oviduct consists of an anterior infundibulum, middle uterus, and posterior vagina (Fox, '63; Fig. 5). Gross anatomy of the oviduct of *A. carolinensis* is similar to the general description for lizards of Cuellar ('66a); the histology of the oviduct is as described by Fox ('63) except that no posterior vaginal pouch was

found. The posterior half of the vagina of *A. carolinensis* is folded longitudinally and lined with simple, nonciliated epithelium. The anterior half consists of two parts, the utero-vaginal transition and the area just posterior to the transition, which consists of a thin tube flanked on two sides by openings which extend anteriorly and terminate blindly in the oviducal wall. The entire anterior half of the vagina is highly folded with ciliated and nonciliated cells alternating in the epithelium (Fig. 15). The sperm storage tubules are located in the utero-vaginal transition (Figs. 11, 12).

Relationship of the hemipenis and cloaca in copula

Study of the animals frozen in copula reveals that the male intromits the hemipenis on the side closest to the female (Fig. 2). Sections of the cloaca and hemipenis from frozen pairs show that the hemipenis intromits only into the cloaca and does not enter the oviducts themselves. During copulation, however, the sulcus is placed dorsally in the female so that the two openings of the sulcus line up perfectly with the dorsally-placed openings of the oviducts (Figs. 6-9). No sperm were observed in these sections. Ejaculate moving into the sulcus is visible, however, at the base of the hemipenis in the frozen pairs (Fig. 2). The longitudinal sections also show the cloacal epithelium conforming to the flounces on the surface of the hemipenis.

Sperm transport

Results of the timed sacrifice experiment are summarized in Table 1. Oviducal sections of two specimens fixed 15 and 30 min after termination of mating show the sperm mass extending anteriorly from the cloaca through the posterior half of both vaginas. The sperm mass is easily observed because the ejaculate appears as a bright pink when stained with hematoxylin and eosin (Fig. 10).

In the two specimens sacrificed two hours after mating, the sperm mass was in the same position as in females sacrificed immediately following mating, but individual sperm had also progressed into the utero-vaginal transition and were observed entering the storage tubules. In more ventral sections of both 2-hr specimens in which the opening of the intestine to the cloaca is visible, the sperm mass extends into both oviduct openings and the intestine (Fig. 10). In both animals, a greater amount of sperm was observed in the intestine than in either oviduct.

In the two 4-hr animals, the sperm mass appears to be more concentrated anteriorly than

before, but less sperm was observed in the utero-vaginal transition than was seen in the 2-hr specimens. Large amounts of sperm were found in the intestine.

By six hours after mating, the sperm mass had disappeared completely, with sperm concentrated in the tubules, the utero-vaginal transition, and the area just posterior to the transition. In female #7, a few sperm were scattered through both uteri and infundibula, and some of the infundibulum sperm appeared altered, with increased head size and smaller tails than normal. In the other female (#8), greater amounts of sperm appeared in the right uterus and infundibulum, including a few sperm clumps, while the left uterus and infundibulum were devoid of sperm. In this animal, one small (< 5.0 mm) follicle was present in each ovary; the male used the left hemipenis in mating. No sperm were observed in the intestine of either six-hour animal.

Little change was seen in the females sacrificed 18 hr after copulation. Sperm was found concentrated in the utero-vaginal transition and storage tubules, and scattered through the uterus and infundibulum. One female (#10) had very little sperm in the left oviduct, however, while the right contained large amounts of sperm. This female had a large follicle (6.3 mm) on the left and a small follicle on the right (3.7 mm); the left hemipenis was used by the male during mating. In the other 18-hr female, the anterior end of the cloaca was blocked by what appeared to be feces. The cloaca lumen was distended and packed with sperm, and sperm was detected in the intestine.

Sperm was found only in the storage tubules and the infundibulum at 24 and 48 hr after mating, with the larger portion of sperm occurring in the tubules.

Sperm storage tubules

Transverse sections of the utero-vaginal transition show sperm storage tubules embedded in all sides of the oviduct wall (Fig. 12). The tubules run approximately parallel to the lumen, but there is much curling and twisting. Longitudinal sections show that the tubules are confined to the transition area, and are not present in any other areas of the oviduct (Fig. 11).

Scanning electron micrographs show the tubules to be discrete structures, highly differentiated from the rest of the oviducal wall (Figs. 13-15). The tubules consist of invaginations into the epithelium that appear to be formed at the end of the longitudinal epithelial folds by a fusion of the tops of the folds (Figs. 13,

ABBREVIATIONS

AO, apical opening	OV, ovaries
CL, cloaca	R, right
CO, cloacal opening	SM, sperm mass
F, female	SS, sulcus spermaticus
HP, hemipenis	UT, uterus
IF, infundibulum	UV, utero-vaginal transition
IT, opening of (severed) intestine	VG, vagina
L, left	

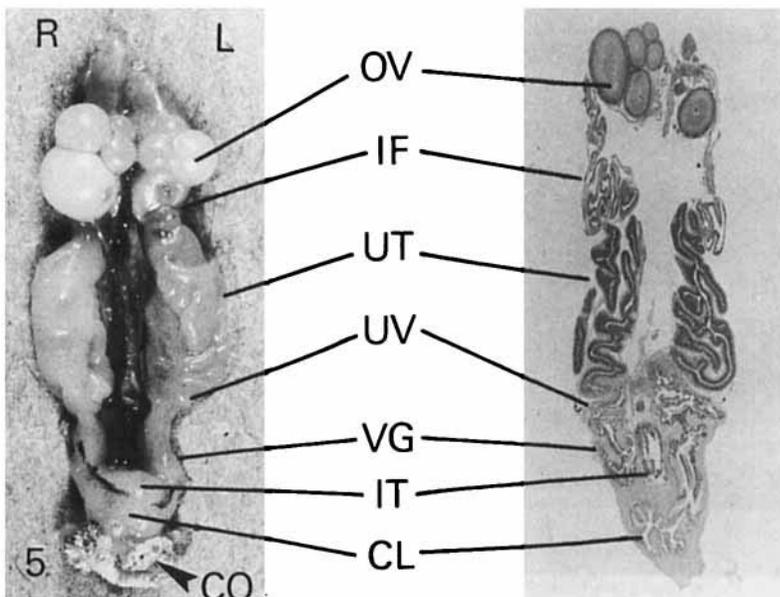
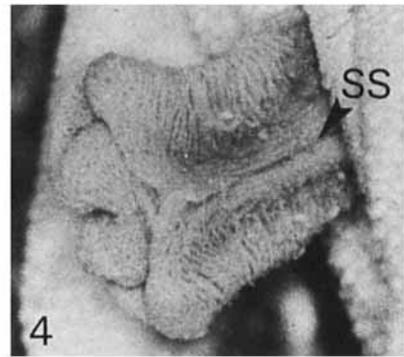
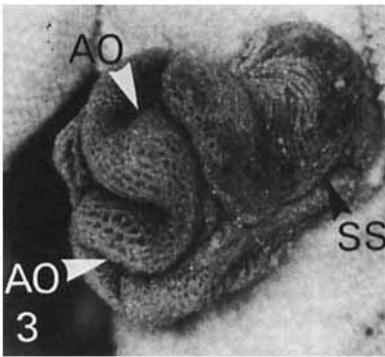
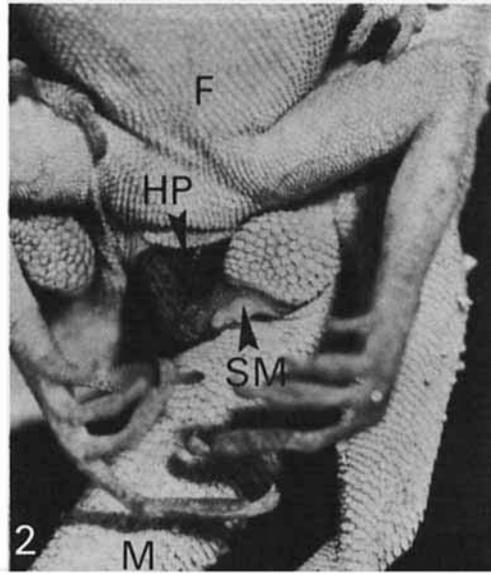
Fig. 1. Mating position of *A. carolinensis*.

Fig. 2. Ventral view of pair frozen in copula. Note sperm mass (SM) moving into the sulcus spermaticus at the base of the hemipenis.

Fig. 3. Apical view of everted left hemipenis. Note calyces and two apical openings (AO) of the sulcus spermaticus (SS).

Fig. 4. Medial view of everted left hemipenis. Note flounces and sulcus spermaticus (SS).

Fig. 5. Ventral views of the entire female reproductive tract of *A. carolinensis*. Left, whole tract; right, longitudinal section (7 μ m) of tract.



ABBREVIATION

OO, oviducal openings



Fig. 6. Longitudinal section of hemipenis and cloaca *in copula*. Note sulcus spermaticus (SS) bifurcating into two apical openings (AO) and apposition of right apical opening with oviducal opening (OO). 40 μm , $\times 31$

ABBREVIATIONS

CE, cloacal epithelium

HS, hemipenile surface



Fig. 7. Longitudinal section of the left side of the same specimen as in Figure 6. Note apposition of left apical opening (AO) with oviducal opening (OO). $40\ \mu\text{m}$, $\times 60$

Fig. 8, 9. Transverse section of right and left side of the hemipenis and cloaca in copula. Note apposition of apical openings (AO) on the hemipenis with oviducal openings (OO) in the cloacal wall and calyces on the hemipenile surface (HS). $40\ \mu\text{m}$, $\times 31$

TABLE 1. Progress of sperm in the genital tract of female *Anolis carolinensis* during the 48 hr following insemination

Animal	Time to sacrifice	Posterior vagina & cloaca	Utero-vaginal transition		Tubules		Uterus		Infundibulum		Intestine
			L	R	L	R	L	R	L	R	
1	15 min	XX	-	-	X	X	-	-	-	-	-
2	30 min	XXX	-	-	X	X	-	-	-	-	XXX
3	2 hr	XXX	XXX	XXX	XX	XX	-	-	-	-	XXX
4	2 hr	XXX	XXX	XXX	XX	XX	-	-	-	-	XXX
5	4 hr	XXX	X	-	-	-	-	-	-	-	XXX
6	4 hr	XXX	XX	XX	X	-	-	-	-	-	XXX
7	6 hr	-	XXX	XXX	XXX	XXX	X	X	X	X	-
8	6 hr	-	XXX	XXX	XX	XX	XX	XX	XX	XX	-
9	18 hr	-	X	X	XXX	XXX	X	X	XX	XX	XX
10	18 hr	-	-	-	X	XXX	XXX	XXX	XX	XX	-
11	24 hr	-	-	-	XXX	XXX	-	-	X	XX	-
12	24 hr	-	-	-	XXX	XXX	-	-	XX	XX	-
13	48 hr	-	-	-	XX	XX	-	-	X	X	-
14	48 hr	-	-	-	XXX	XXX	-	-	XX	XX	-

- ... no sperm
 X ... few sperm
 XX ... moderate amount of sperm
 XXX ... large amount of sperm
 At each time interval after copulation, two females were sacrificed, the urogenital system dissected and prepared for histological examination, and the amount of sperm in each portion of both the left and right (L & R) oviducts recorded. Amounts of sperm given are not absolute; rather, they are estimates relative to all specimens studied.

16-18). This arrangement could serve to funnel sperm into the tubules.

Approximately 75-150 tubules are present in each oviduct. Results of the sperm counts and tubule measurements are summarized in Table 2. Numbers of sperm contained in each tubule varied from 0 to 633, with an average of 319. Tubules ranged from 17.4 to 46.4 μm in total width, 5.8-23.2 μm in lumen width, and 52-581 μm in length. No relationship was observed between tubule position and number of sperm in the tubule.

Sperm were observed entering the tubules with heads oriented toward the distal end of the tubule, and a similar orientation was observed in longitudinal sections of storage tubules (Fig. 19). In transverse section, sperm appeared to be arranged in whorls inside the tubules. No attachment of the sperm heads to the tubule epithelium was observed. In scanning electron micrographs of animals sacrificed five hours after insemination, sperm appeared concentrated at the base of the folds in the utero-vaginal transition (Fig. 15).

DISCUSSION

Sperm transport in the female genital tract has been studied in humans (reviewed in Croxatto et al., '74), nonhuman primates (reviewed in Hafez and Jaszczak, '74), livestock (reviewed in Robinson, '74; Hunter, '74; Thibault et al., '74; Hawk '74), rabbits (Lambert and Tremblay, '78; Asch et al., '78), rats (Matthews and Adler, '77, '78; Shalgi and Kraicer, '78), poultry (Howarth, '70), and amphibians (reviewed by Boisseau and Joly, '74). Sperm transport has never been systematically studied in a reptile, although some observations have been made from histological examinations of oviduct sections (Saint-Girons, '75).

Extended sperm storage (more than two days) in the female genital tract has been reported in mammals (bats, reviewed in Racey, '75; dogs, Doak et al., '67; horses, Burkhardt, '49), birds (reviewed in Howarth, '74), reptiles (reviewed in Saint-Girons, '75), amphibians (reviewed in Boisseau and July, '74), and fishes (reviewed in Howarth, '74). In lizards, there are many reports of fertile eggs laid by females isolated from males (Atsatt, '53; Church, '62; Cuellar, '66b). True seminal receptacles are found only in the Chamaeleonidae (Saint-Girons, '62; Veith, '74) and the genus *Anolis* (Fox, '63). Sperm is stored in epithelial crypts in the Agamidae and nonanoline Iguanidae (Cuellar, '66a). Sperm may also be stored in the lumen as in snakes, Gekkota and Scincomorpha (King, '77; Yaron, '72; Schaefer and

Roeding, '73). The present study is the first to study the mechanism of sperm transport and storage in a lizard.

Hemipenile-cloacal relationship in copula

The hemipenile-cloacal sections of *A. carolinensis* pairs frozen in copula demonstrate that the two apical openings of the bifurcate sulcus on the hemipenis are apposed to the openings of the oviducts in the cloaca. This arrangement is similar to that described by Pope ('41) who studied a pair of snakes (*Liophis poecilogyris*) killed and fixed in copula. Due to the absence of sperm in the hemipenile-cloacal sections in the present study, the details of the mechanism of sperm transfer remain in question. This absence of sperm may be due to the method of preparation. Alternatively, this finding may indicate that sperm transfer takes place late in copulation as specimens were frozen within five minutes after intromission; mean mating duration of *A. carolinensis* is 9.22 min (Valenstein and Crews, '77).

Pope ('41) proposed that the gross shape of the hemipenis was directly related to the shape of the cloaca, giving examples of species with bilobed hemipenes and correspondingly bilobed cloacae. Ludwig and Rahn ('43), from observations of the size and shape of the hemipenis and vagina of *Crotalus viridis viridis*, suggested that the two lobes of the highly divided hemipenis were inserted into each vagina during copulation. In a dissection of a pair of snakes (*Heterodon platyrhinos*) preserved in copula, Edgren ('53) found that the hemipenis only filled three-quarters of the cloaca, with a mass of ejaculate occupying the anterior quarter. Edgren concluded that the shapes of the hemipenis and cloaca do not always correspond closely. This evidence is weak, however, because the hemipenis in the specimen studied was partially retracted. Inger and Marx ('62) found no correlation between the shape of the cloaca and the type of hemipenis in the snake *Calamaria lumbricoidea*, taking note that this was one of the most widely distributed species in the genus. Also, in a study of the systematics and evolution of the snake genus *Natrix*, Malnate ('60) concluded that genera with the simple sulcus were more advanced evolutionarily than those with a forked sulcus.

While the bifurcate sulcus on the hemipenis of *A. carolinensis* seems to be an elegant system for directing the sperm to the oviducts, this species does have a simple (nonbilobed) hemipenis, and the large sperm masses observed in the intestine after mating clearly indicate significant gametic wastage. Anoles of the *A.*

intermedius species group have a bilobed hemipenis-cloaca system coupled with a bifurcate sulcus (Crews and Miyata, unpublished data). It would be of interest to determine if these species have reduced gametic wastage.

Sperm transport and storage

The only published discussion of sperm transport in reptiles is in a review article by Saint-Girons ('75) and is based on casual observations. The present study demonstrates that the movement of sperm from the cloaca up the vagina is rapid and probably active since no cilia were observed in the posterior vagina. The observation in the present study of sperm entering the sperm storage tubules and their orderly arrangement within the tubules support the hypothesis of active transport and is in agreement with previous studies (Fox, '63; Cuellar, '66a; Hattan and Gist, '75; King, '77). Neither the ovarian state of the female nor the choice of hemipenis used by the male appear to affect sperm transport, as no correlation between these parameters was found in this study. Saint-Girons ('75) speculates that active sperm movement is oriented by a concentration gradient of oviducal secretions. Both Fox ('56) and Hoffman and Wimsatt ('72) suggest that the cilia in the openings of sperm storage ducts could produce an outward current for the sperm to swim in against. The formation of the tubules by fusion of longitudinal folds and the concentration of sperm at the bottom of the folds suggest another mechanism for transport of sperm into the tubules; that is, by traveling along the folds, sperm may be funneled directly into the tubules. Saint-Girons ('75) proposed that, as in mammals, the sperm stops in the vagina, and the migration from the vagina to the ovaries was separate and "as rapid and massive" as the movement directly following insemination. This does not appear to be the case in *A. carolinensis*, because small amounts of sperm are found in the infundibulum within six hours after mating. Saint-Girons favors an active transport to the infundibulum, and the evidence from the present study of *A. carolinensis* tends to support this, as the uterus is not ciliated and sperm are usually observed in the lumen of the uterus. This evidence is weak, however, and artifacts due to fixation are possible.

The question of how sperm stored in the tubules are released to fertilize the ovum remains unsolved. Fox ('56) suggested that the pressure of the ovum passing the infundibular receptacles in snakes would force sperm out. This system would not work in *A. carolinensis*,

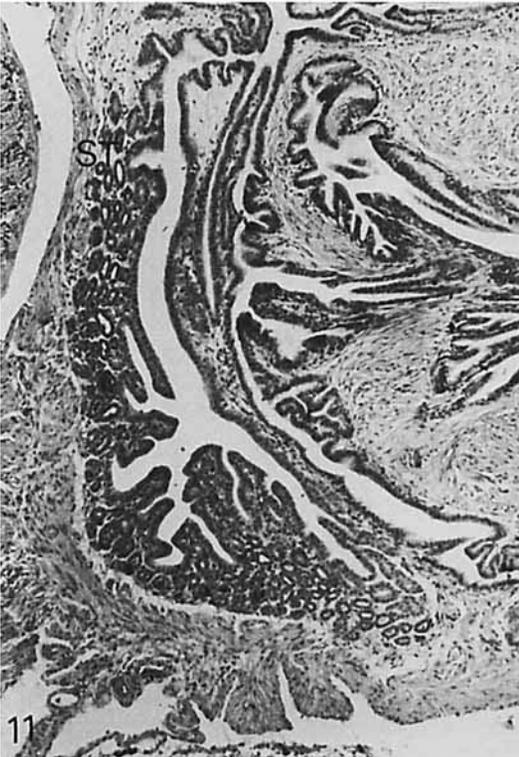
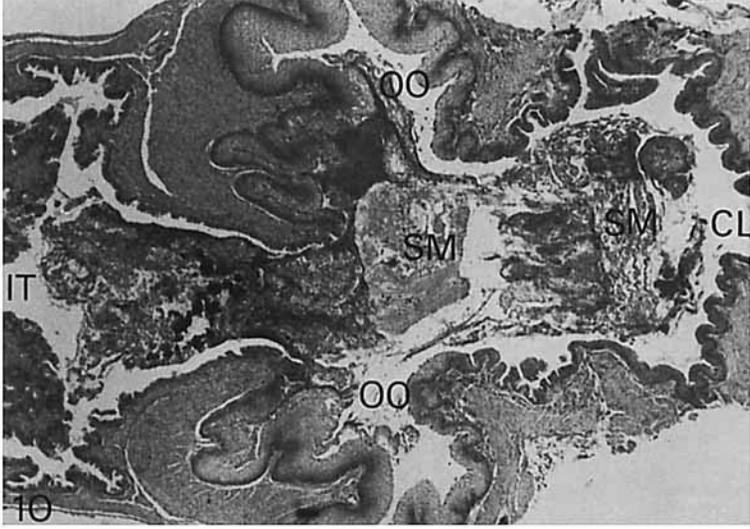
ABBREVIATIONS

LG, longitudinal view	ST, sperm storage tubules
LU, lumen of oviduct	TR, transverse view
SB, sperm bundles	

Fig. 10. Longitudinal section through the posterior 3 mm of the reproductive tract of a female sacrificed 4 hr after insemination. Note sperm mass (SM) extending through the cloaca (CL) and into the intestine (IT) and both oviducal openings (OO). 7 μm , \times 31

Fig. 11. Longitudinal section through the utero-vaginal transition. Note arrangement of sperm storage tubules (ST), some containing dark-staining sperm bundles (SB). 7 μm , \times 60

Fig. 12. Transverse section through the utero-vaginal transition. Note sperm storage tubules arranged around the entire oviducal wall. Some tubules appear in transverse section (TR), some longitudinal (LG), and most contain sperm bundles (SB). 10 μm , \times 80



ABBREVIATIONS

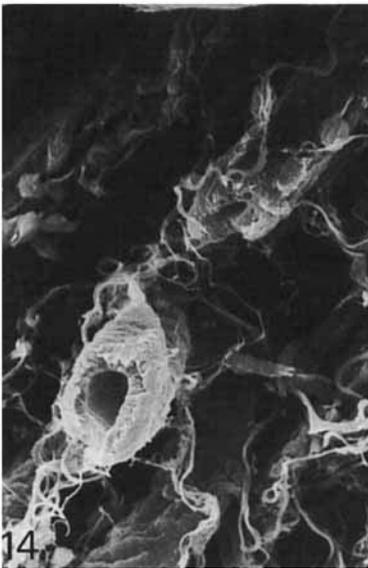
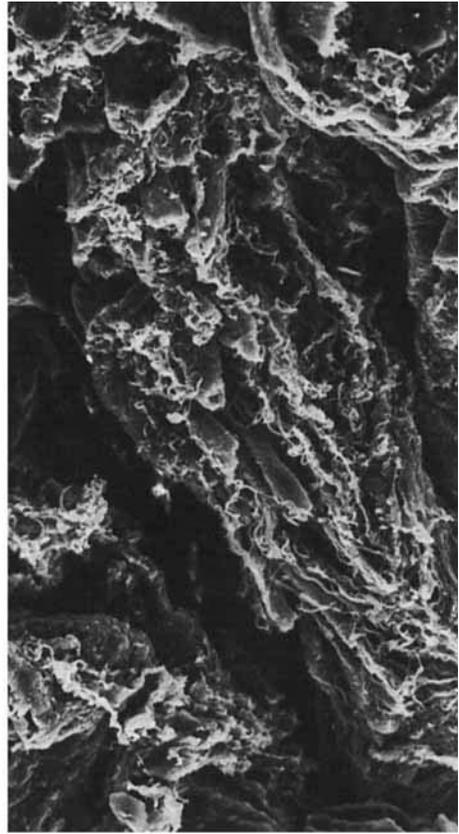
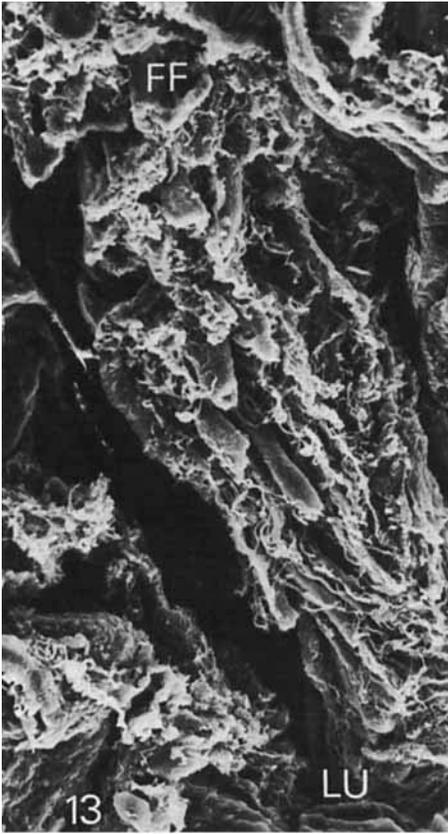
FF, fold fusing

CI, cilia

Fig. 13. Stereo pair scanning electron micrographs of tubule area inside oviduct wall. Lumen (LU) runs from lower left to upper right. Several sperm storage tubules (ST) appear with ends cut off. Note fold fusing (FF) into tubule middle left. (Stereo pair should be viewed from either the left or right sides using stereo glasses.) $\times 200$

Fig. 14. Scanning electron micrograph of two sperm storage tubules in the wall of the oviduct. Ends of tubules are cut off, exposing the tubule lumen. Tubules are surrounded by connective tissue. $\times 1,000$

Fig. 15. Scanning electron micrograph of the lumen of the utero-vaginal transition of a female sacrificed 5 hr after insemination. Sperm are congregating at the bottom of the fold, and one sperm bundle (SB) appears at the opening of a cleft. Note the alternation of ciliated (CI) and nonciliated cells. $\times 900$



however, as the egg is already shelled by the time it enters the vagina where the sperm storage tubules are located (Fox, '63; Cuellar, '66a). Cuellar ('66a) found an aggregation of sperm receptacles adjacent to the smooth muscle band of the oviduct and suggested that contractions accompanying ovulation could serve to force sperm out. Saint-Girons ('75) believes that muscle contractions alone are insufficient, and suggests that chemical stimulation may also be necessary. The receptacles in *A. carolinensis* are not aggregated near the muscle band, but are arranged on all sides of the oviduct wall. Still, muscle contractions accompanying ovulation is a promising hypothesis to explain tubule evacuation.

Importance of sperm storage in life history of A. carolinensis

According to Hamlett ('52), in *A. carolinensis* "it seems likely that there is a separate insemination for each egg ovulated rather than any prolonged storage of spermatozoa in the female tract (p. 185)." Gordon ('56) isolated female *A. carolinensis* prior to the breeding season, and found all eggs laid to be infertile. If isolated during the breeding season, females laid one fertile egg with all subsequent eggs being infertile. However, as no information on female size is provided, it is possible that these females were reproductively immature during the previous fall and therefore were not inseminated. Fox ('63) found sperm in the sperm storage ducts of females captured before the start of the breeding season and therefore concluded that the sperm had been stored at least seven months. Fox also reports two females with fertile eggs at the beginning of the season, with sperm present only in the sperm storage tubules. Cuellar ('66b), in a systematic study of delayed fertilization in isolated female *Uta stansburiana*, found that the first clutch was 95% fertile, the second 53% fertile, and the third infertile. Licht ('73), however, reported two isolated female *A. carolinensis* that laid fertile eggs in the laboratory seven months

after the end of the breeding season; one of these females had previously laid five infertile eggs. It is clear from these findings that in *A. carolinensis*, the tubules protect the stored sperm during the passage of an egg through the vagina.

Fox ('63) suggested that the ecological function of sperm storage in *A. carolinensis* was to lengthen the breeding season by fertilizing eggs ovulated early in the season (before mating takes place) and late in September (after male testicular regression occurs in August) (see also Smyth and Smith, '68). Sperm storage would also be valuable in cases in which males are temporarily absent (due to territorial disruption or predation) when the female is in estrus. Furthermore, the unique reproductive pattern of the anoles (Smith et al., '72) make sperm storage potentially more useful in this genus than in other lizard genera. An adult female *A. carolinensis* lays one egg every 10 to 14 days (Hamlett, '52; Crews '73a; Licht, '73) and would have to mate that often if sperm were not stored. Although matings are well timed to coincide with ovulation (Crews, '73a), the length and location of mating exposes the females to predation (Crews, '73b). Thus, sperm storage in female *A. carolinensis* could operate to increase the reproductive success of females in two ways: 1) increased fecundity (longer breeding season, reduced dependence on male populations); and 2) increased female survivorship (reduction of predation during mating).

Sperm storage could also function to enhance the ability of *A. carolinensis* to colonize insular habitats, as only one female, rather than a mating pair, is necessary as a founder. Williams ('69) found *A. carolinensis* to be highly successful in colonizing the islands of the West Indies. However, experimental evidence indicates that observation of male courtship is necessary for normal ovarian function and egg-laying in *A. carolinensis* (Crews et al., '74). Further study is necessary before the adaptive significance of sperm storage is clearly understood.

Figs. 16-18. Sequence of serial, transverse sections through the utero-vaginal transition showing the beginning of a tubule formed from the bottom of a fold.

Fig. 16. Thin fold (FF) visible with two tubules nearby (ST). Lumen (LU) is at right.

Fig. 17. Epithelium at top of fold has fused and the lumen of the tubule is now visible.

Fig. 18. Tubule epithelium is now separate from the fold epithelium; new tubule (ST) is visible. 10 μm , \times 500

Fig. 19. Longitudinal view of a sperm storage tubule (ST) and the fold (FF) leading into the tubule from a female sacrificed 6 hr after insemination. Sperm are visible in the fold with heads oriented distally (toward the tubule). Note also the orientation of sperm in tubule. 7 μm , \times 500

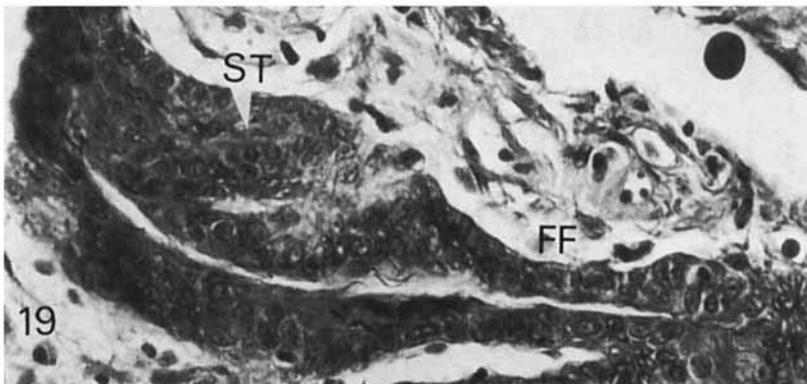
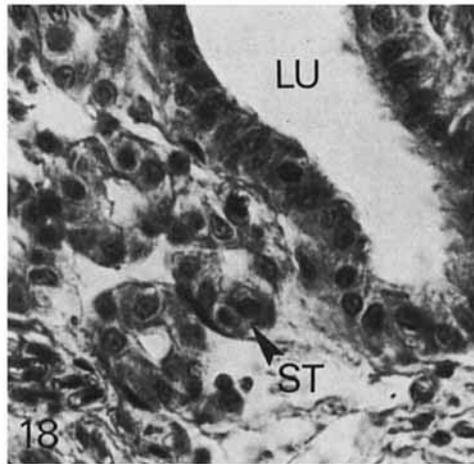
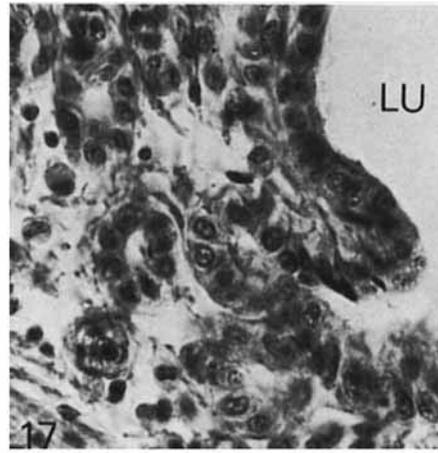
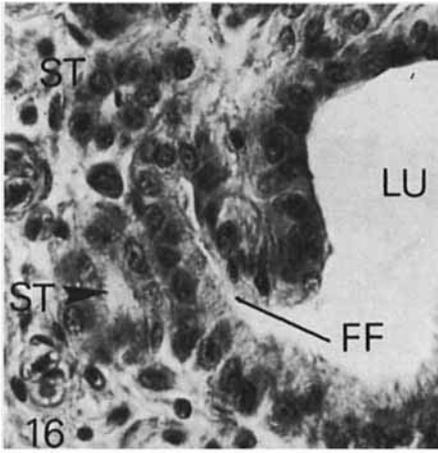


TABLE 2. Average number of sperm contained in five sperm storage tubules from the left and right oviducts of two *Anolis carolinensis* sacrificed 48 hr after insemination (ranges of these measurements also provided)

	Left oviduct					Right oviduct				
	1	2	3	4	5	1	2	3	4	5
Lizard 13:										
No. of sperm per tubule	105.67	274.33	336.33	59.34	0	326.66	6.00	23.00	5.67	622.33
range: low	100	253	302	56	0	304	6	22	5	579
high	112	308	373	64	0	352	6	24	6	666
Tubule size (μm):										
lumen width	23.2	11.6	11.6	5.8	11.6	11.6	11.6	11.6	11.6	20.3
total width	40.6	29.0	46.4	23.2	26.1	34.8	29.0	23.2	26.1	34.8
total length	98	49	126	35	91	217	196	210	112	259
Lizard 14:										
No. of sperm per tubule	288.67	415.67	627.34	472.67	521.00	402	319.67	453.34	484.67	633.67
range: low	272	376	563	429	469	364	291	417	419	562
high	310	452	692	512	584	441	348	497	554	716
Tubule size (μm):										
lumen width	11.6	5.8	11.6	5.8	14.5	8.7	8.7	11.6	11.6	14.5
total width	34.8	29.0	34.8	17.4	29.0	23.2	29.0	23.2	23.2	29.0
total length	343	322	238	581	210	231	182	203	203	196
Average no. of sperm per tubule										
Left oviduct	155.13									
Right oviduct		196.73								
overall			175.93							
Lizard 13										
Lizard 14	465.07		458.67							
Lizard 13 + 14				461.87						
					318.90					

Average dimensions of tubules (μm) also given.

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