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STORAGE OF SPERM IN THE REPTILIAN OVIDUCT

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Abstract

Female reptiles have the ability to store sperm within their reproductive tracts for extended periods of time. Sites of sperm storage in lizards and snakes include both the anterior vagina and the infundibulum. Vaginal receptacles are found in between longitudinal folds (snakes) and in tubules formed by invaginations of the epithelium (lizards). Infundibular receptacles are alveolar or tubular in structure and are formed from invaginations into the lamina propria of the oviduct wall. In turtles, sperm are stored in the posterior portion of the tuba, in tubular albumen-secreting glands. Sperm may be embedded within the cells of the receptacles, but the membranes of each remain intact. The morphology of the receptacles is characteristic of the normal glands of the area.

Key Words: Reptilia, oviduct, sperm storage, seminal receptacles, delayed fertilization, histology.

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Introduction

The storage of sperm within the female reproductive tract for extended periods of time following mating is a phenomenon which is found in all but the most primitive vertebrate groups. Sperm storage, which prolongs the period of sperm viability and thus the capacity for fertilization, can provide a reproductive advantage for those species whose ovulatory cycles are not precisely synchronized with environmental or social stimuli, and for those in which male and female gametes do not mature at the same time.

In many species, sperm storage within the female has been inferred from observations of delayed fertilization in which viable offspring are produced by females isolated from males for periods of time exceeding normal gestation or vitellogenesis. Using this criterion, sperm may be stored for as few as 5 - 6 days in the horse (see Howarth, 1974) to 7 years in the snake Acrochordas javanicus (Mangusson, 1979). As a group, the reptiles are capable of storing sperm for periods of time longer than other vertebrates, and estimates using the above criterion range from two months to 7 years. However, the use of delayed fertilization as definitive evidence for sperm storage in reptiles has been criticized by Fox (1977) based on the fact that some species of lizards are parthenogenic. Other methods used to establish sperm storage include oviductal flushings following isolation from males and the observation of sperm in histological preparations of the oviduct. It must be emphasized that each of these methods is insufficient to demonstrate that stored sperm are, in fact, capable of fertilization. Techniques of establishing the potency of reptilian sperm have yet to be developed, and at present one can only assume that stored sperm, by virtue of their morphological integrity, have the potential for fertilization.

Reptiles remain one of the least studied vertebrate groups, and only a few papers have appeared since Saint Girons (1975) reviewed the disposition of sperm within the reptilian oviduct. The present review will concentrate on the anatomy of sperm containing structures within

the oviduct and the implications of sperm storage on reptilian reproduction.

#### Anatomy of the Oviduct

In order to appreciate differences existing in the location of reptilian sperm storage structures, it is useful to review the anatomy of the reptilian oviduct. The account presented here is based on our own studies on turtles as well as several reviews and descriptions of others (Giersberg, 1923; Saint Girons, 1975; Fox, 1977; Halpert et al, 1982). The oviduct consists of the vagina, uterus, tuba, and infundibulum. The oviduct is surrounded by inner circular and outer longitudinal smooth muscle layers, which increase in thickness posteriorly. The epithelium lining the oviduct is simple columnar, consisting of both ciliated and nonciliated cells. The mucosa is thrown up into folds which extend into the lumen; variations in the height of the mucosal folds, and that of their underlying submucosa, form the anatomical basis for the various regions of the oviduct. When present, multicellular glands occupy the lamina propria; it is the presence of such glands in the tuba region which accounts for the major differences in oviductal anatomy among reptile groups.

The snake oviduct (Figure 1A) is similar to that of lizards; some species of snakes possess only a single oviduct (Fox and Dessauer, 1962). The posterior vagina, or vaginal pouch, opens to the cloaca and is usually enlarged; anteriorly the vagina decreases in thickness and forms a short, straight tube. The vagina accounts for less than one third the length of the oviduct in the garter snake, *Thamnophis sirtalis* (Halpert et al. 1982). The vaginal mucosa is thrown up into longitudinal folds; these are quite high in the vaginal pouch, but become shorter and more flattened anteriorly. The vaginal epithelium contains mucus-secreting cells (Ludwig and Rahn, 1943).

In lizards (Figure 1B), the vagina comprises less than one fourth the length of the oviduct, the uterus accounts for approximately two thirds, and the tuba and infundibulum above, the remainder. The posterior part of the vagina is convoluted and swollen, in part due to the increased thickness of the circular muscle layer. Fox (1963) refers to this enlargement as a vaginal pouch in the green anole (*Anolis carolinensis*), although Conner and Crews (1980) were unable to confirm its existence. Anteriorly, the vagina narrows somewhat to a straight tube. The mucosa of the vagina is thrown up into numerous longitudinal folds. These vary in their height and number depending on the species. In both lizards and snakes, sperm storage structures are found in the furrows between the longitudinal mucosal folds of the anterior portions of the vagina. Because these structures are found only in the anterior vagina, they have been referred to as the vaginal receptacles.

The transition from the vagina to the uterus in both lizards and snakes (Figures 1A, B) is characterized by an abrupt decrease in the height and number of the mucosal folds. The entire

uterus is highly convoluted in lizards, but in snakes the convolutions are conspicuous only at the anterior end. In both groups, the uterus is the major segment of the oviduct, and secretes both the egg shell and shell membranes. The luminal surface of the uterus is flattened with low, irregular mucosal folds. The lamina propria of the uterus contains tubuloalveolar glands which discharge their secretions to the lumen via short ducts. These glands hypertrophy during the egg-laying season, and account in large part for the increase in oviduct weight during that time. The secretory product of these tubuloalveolar glands is believed to be mucoid on the basis of histochemical criteria (Fox, 1956). The identity of the secretory product of the nonciliated secretory cells of the uterine epithelium is unknown.

The tuba region of the oviduct in lizards and snakes is a short, convoluted segment connecting the uterus to the infundibulum. The tuba is presumed to secrete the albumen proteins which separate the vitelline membrane from the shell membranes of the squamate egg (Packard et al. 1977). However, glands are generally absent from this region of the oviduct in lizards and snakes (Saint Girons, 1975), and the source of the small quantities of albumen present in the eggs of squamate reptiles remains unknown.

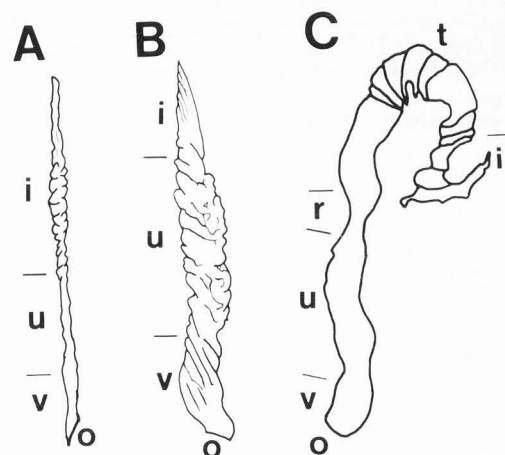


Figure 1. Diagrammatic representation of the, A) snake, B) lizard, and C) turtle oviducts. Legend: v. vagina; u. uterus; t. tuba; r. transition region; i. infundibulum; o. opening to cloaca.

The wall of the infundibulum in both groups is thin due to the absence of multicellular glands occupying the lamina propria. The epithelium consists of a high proportion of ciliated cells and is thrown into short, narrow folds which protrude into the lumen. Sperm storage areas may be formed from tubules or alveoli branching off from the grooves in between the folds; these will be referred to as

infundibular receptacles. The oviduct of the rhynchocephalian lizard Sphenodon punctatus has been described (Gabe and Saint Girons, 1964) and resembles that of other lizards.

The turtle oviduct (Figure 1C) varies from that of lizards and snakes in that the tuba region is extensive and contains tubular glands. The vagina is short and has pronounced longitudinal folds with secondary and tertiary branches. The uterus comprises approximately 30 per cent of the length of the box turtle (Terrapene carolina) oviduct (Hattan, 1971). The uterine mucosa is thrown into irregular folds. The lamina propria beneath the uterine epithelium is packed with tubular glands, which open to the lumen via short ducts. The uterus is separated from the tuba by a short region devoid of glands which Giersberg (1923) identified as the transition to the uterus. In the box turtle, it is about 1 cm in length. Mucosal folds of this region are tightly packed, reduced in height, and are flattened at the apex (see Figure 6). The wall of the transition region is thin and translucent due to the absence of glands from the lamina propria.

The tuba region begins abruptly with a broadening of the mucosal folds and their extension into the lumen. The folds assume a longitudinal orientation with major grooves separating them. The lamina propria of these folds is occupied by tubular glands; at the interface of the tuba to the transition region, these glands are few in number, and connect with the lumen via ducts composed of epithelial cells. The ducts are found most frequently in the grooves between the folds but may exist anywhere on the mucosal folds. Anteriorly, the folds broaden and form longitudinal ridges running the length of the oviduct; they may have secondary branches, giving rise to both major and minor grooves between mucosal folds. The lamina propria of the folds is occupied by extensively-branched tubular glands; these radiate from the luminal epithelium to the base of the lamina propria, and there run parallel with the axis of the oviduct. In the midportion of the tuba, these glands completely fill the lamina propria. The tuba region of the oviduct is highly convoluted in turtles and extensive; it comprises approximately two thirds of the oviduct length.

The tuba of the turtle oviduct is similar in morphology and function to the magnum of the avian oviduct (Aitken and Solomon, 1976). Turtle eggs contain substantial quantities of albumen which are released from these tubular glands (Aitken, 1971; Solomon, 1983), and Giersberg (1923) and others have identified these glands as albumen-secreting glands. The albumen of turtle eggs, unlike that of birds, is of uniform viscosity, and this has prompted some investigators (Solomon and Baird, 1979) to suggest that reptilian albumen is more homogeneous than its avian counterpart, which consists of a large number of secreted proteins (Gilbert, 1971). There are no reported analyses of reptilian albumen either secreted by the oviduct or present in the egg. Sperm-containing tubules are found in the posterior terminus of

this large glandular region; since they are unspecialized (see below), they will be referred to as albumen receptacles (Hattan and Gist, 1975).

Anterior to the tuba, the mucosal folds become narrow and shallow, and the lumen wider as the albumen glands dissipate. The lamina propria of the infundibulum is devoid of glands, and the mucosa is characterized by an even greater reduction in both the height and number of the folds. Ciliated cells of the infundibulum epithelium are clumped together among the more numerous nonciliated cells.

The oviductal anatomy of crocodylians is similar to that of the turtle, possessing separate albumen and shell-forming areas. Detailed descriptions of the adult oviduct are not available.

#### Sperm receptacles

There is a paucity of information on the anatomy of reptilian sperm storage structures; most of our knowledge is based on only a few studies on even fewer species. Nevertheless, the oviductal structures which are utilized for sperm storage in reptiles are characteristically unspecialized structures except for the presence of stored sperm.

#### Vaginal receptacles

Vaginal sperm storage structures (Figure 2) are found in lizards and snakes (Ludwig and Rahn, 1943; Fox, 1963; Cuellar, 1966a; Schaefer and Roeding, 1973; Halpert et al., 1982). Receptacles in the red-sided garter snake (T. sirtalis) were recently examined by Halpert et al. (1982). They are simple in morphology and consist of the terminal portions of longitudinal "furrows" which separate the numerous mucosal folds protruding into the lumen of the anterior vagina (Figure 2b). The furrows are approximately 1 cm in length and are lined by PAS-positive ciliated and nonciliated cells identical to those lining the vaginal lumen. In animals collected in the fall, sperm occupying these receptacles have their heads directed away from the vaginal lumen, and appear in contact with the epithelium. Halpert et al. (1982) suggest that the PAS-positive secretions of the epithelium lining the furrows provide nutrition for the stored sperm. They also hypothesize that sperm from fall matings are stored in these uterovaginal furrows and migrate or are transported up the oviduct to the infundibular receptacles (see below) over winter. The overwintering of sperm within the oviduct corroborates the results of earlier workers in this (Rahn, 1940; Fox, 1956) and other (Ludwig and Rahn, 1943) species of snakes where sperm were observed prior to spring mating in flushes from and in histological sections of the posterior portions of the oviduct.

In lizards, the sperm receptacles are also derivatives of the anterior vagina (Cuellar, 1966a; Fox, 1963; Saint Girons, 1962; Conner and Crews, 1980). As in snakes, sperm storage areas in lizards are formed from grooves in between the longitudinal mucosal folds of the anterior

vaginal wall (Figure 2a). The receptacles in lizards consist of short tubules formed from invaginations of the epithelium. Thus, they form tubules in the lamina propria which run parallel to the grooves from which they were formed. The tubules may extend anteriorly into the uterine region beneath the epithelium. The tubules are not straight, but frequently are coiled, sometimes branched, and may be seen extending into the lamina propria and between the muscle layers (Cuellar, 1966a). The epithelium comprising the storage tubules consists of ciliated and nonciliated cells, although the number of ciliated cells decreases in the peripheral ends of the tubules where sperm are aggregated.

Sperm within these tubules are oriented with their heads towards the terminus of the tubule. Cuellar (1966a) observed a close association between stored sperm and the epithelium of the tubules. The duration of sperm storage in the lizard receptacles is variable. Cuellar (1966b) estimates that sperm were stored by the lizard *Uta stansburiana* for almost three months. Fox (1963) found stored sperm in the receptacles of *A. carolinensis* following seven months of isolation.

Conner and Crews (1980) have studied the time course of sperm movement up the oviduct of *A. carolinensis* following copulation. Sperm were found in the vaginal receptacles within 2 h following copulation, and in the infundibulum by 6 h. By 24 h, sperm had vacated all areas of the oviduct except the infundibulum and the vaginal receptacles. They concluded that storage of sperm in the green anole allows the females to ovulate (a single egg is produced every 10 to 14 days during the breeding season) repeatedly without the need for additional matings. The receptacles of *A. carolinensis*, located in the submucosal connective tissue, provide an isolated environment for sperm storage. While this species is unusual in its pattern of ovulation and oviposition, sperm storage could provide the same benefit for all lizards laying multiple clutches during the period of reproductive activity.

The question of how sperm survive during storage in the vaginal receptacles has not been addressed. The close association of sperm with the receptacle wall has led investigators to suggest that sperm derive nutrition from the epithelium or its secretions (Cuellar, 1966a; Halpert et al, 1982). The possibility that metabolism and/or activity of sperm within the tubules might be suppressed, effectively prolonging the life of the stored gametes, seems equally plausible.

If sperm stored in the vaginal receptacles are to be utilized for fertilization, they must relocate to the infundibulum at or near the time of ovulation. While sperm stored in the vaginal receptacles of snakes can easily move (or be moved) from the receptacles to the infundibulum (Halpert et al, 1982), the movement of sperm from the lizard receptacles is more circuitous; sperm must vacate the storage tubules posteriorly to the vagina, and thence move anteriorly towards the infundibulum across a sparsely ciliated

uterine epithelium. How this transport is achieved is not known. Cuellar (1966a) has noted that the vaginal receptacles in several lizards are located in juxtaposition to a smooth muscle band running along the medial edge of the oviduct from the vagina to the ostial opening of the oviduct. He speculates that contraction of this muscle might aid sperm evacuation from the receptacles. While this muscle is not present in all species (Conner and Crews, 1980), oviductal contractions associated with copulation, ovulation, or other stimuli could provide an attractive hypothesis for sperm evacuation from vaginal receptacles.

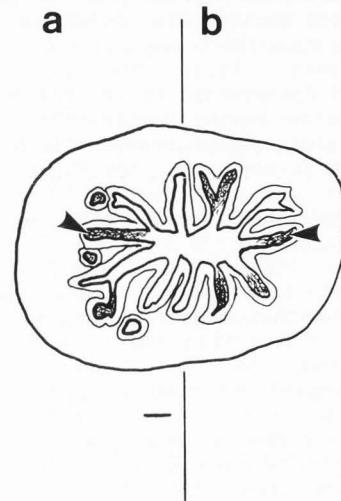


Figure 2. Diagrammatic cross section of the vaginal sperm receptacles found in a) lizards, and b) snakes. Arrow: receptacles containing sperm. Bar = 0.1 mm.

#### Infundibular receptacles

Sperm storage within the infundibulum of the lizard oviduct is not widespread (Figure 3). Cuellar (1966a) found infundibular receptacles in only two of eleven iguanid species examined;

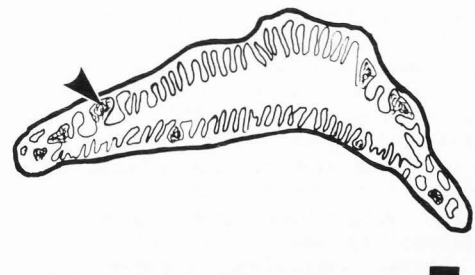


Figure 3. Diagrammatic representation of infundibular sperm receptacles in both lizards and snakes. Arrow: receptacles containing sperm. Bar = 0.1 mm.

neither of the two contained sperm. Conner and Crews (1980) made no mention of infundibular receptacles in their study of *A. carolinensis*, and sperm were absent from the infundibulum of the skink *Eumeces egregius* (Schaefer and Roeding, 1973).

Tubules containing sperm were identified in lizards representing three different families. In both the Gekkonidae and the Eublepharidae, tubules containing sperm were identified on the lateral and medial sides of the oviduct close to the mesenteries and smooth muscle band supporting the oviduct (Cuellar, 1966a). From the published micrographs, the tubules appear to be composed of ciliated epithelial cells not distinguishable from those lining the infundibular lumen. Interestingly, the two lizard species possessing infundibular receptacles with sperm did not possess vaginal receptacles.

The structure of the infundibular receptacles of the lacertid *Acanthodactylus scutellatus* was described by Bou-Resli et al (1981). Tubules containing sperm were located between the mucosal folds of the infundibulum. The tubules were lined by large cells ciliated at the luminal opening but nonciliated towards the blind end of the tubule. These cells were connected to each other via desmosomes and cytoplasmic membranes, and contained electron-dense vesicles in their apical cytoplasm. Sperm were observed in between these cells and embedded within them. Both sperm and the tubule cells, however, retained intact membranes. They suggested that the electron-dense vesicles might serve a nutritive function and that sperm were retained in these tubules in excess of four months.

The infundibular receptacles of snakes are among the best studied. They can be found in many species (see Saint Girons, 1975), but detailed studies of their morphology and their role in reproduction are limited to the genus *Thamnophis*. They were first described by Fox (1956) in *T. sirtalis* and *T. elegans*. These receptacles differ from the vaginal receptacles in that they are alveolar in form and open to the oviduct lumen via short, ciliated ducts (Figure 3). They protrude into the lamina propria of the thin infundibular wall and, when filled with sperm, they protrude as bulges in the oviduct wall (Fox, 1956; Hoffman and Wimsatt, 1972).

The histology of these alveolar receptacles has been described by several investigators (Fox, 1956; Fox and Dessauer, 1962; Fawcett et al., 1972; Hoffman and Wimsatt, 1972). The alveoli are lined by a single layer of nonciliated cells, and open to the lumen via narrow ducts; frequently several alveoli will connect to the lumen via a single duct. The epithelium of the receptacles varies considerably with the ovulatory cycle and with the presence of stored sperm. When empty or as ovulation approaches, the cells lining the receptacles enlarge and become highly vacuolated. In the presence of sperm the cells become more cuboidal and the vacuolation is reduced. These cells have no obvious surface specializations, but do contain many granular inclusions, and possess numerous lipid droplets in the basal portions of the cell. Hoffman and Wimsatt (1972)

speculated that this lipid might provide nutrition for stored sperm based on their findings that stored sperm possessed lipase activity.

Ultrastructural studies confirm an intimate association between stored sperm and the receptacle wall. Hoffman and Wimsatt (1972) found heads of sperm deeply embedded in the receptacle epithelium; however, the membranes of each remained intact, and no surface specializations on either cell were observed. Although sperm embedded within the receptacle cells are apparently normal, receptacle cells, even with sperm in them, possess numerous vacuoles and cytoplasmic inclusions suggestive of autolytic activity.

Infundibular receptacles in snakes are not permanent reservoirs for sperm. Fox (1956) observed that infundibular receptacles of garter snakes collected in summer and autumn were devoid of sperm, even though sperm were present in the lower oviduct. Large amounts of stored sperm were found in the infundibular receptacles of spring caught animals, and Fox (1956) concluded that the infundibular receptacles were filled as the result of spring copulations. Halpert et al (1982) examined the annual movements of sperm within the oviduct of the garter snake, and concluded that while sperm from fall copulations were moved to the infundibular receptacles over winter, they were displaced by sperm inseminated the following spring. According to their hypothesis, sperm from fall copulations stored in the infundibular receptacles would be used for fertilization only if females failed to mate in the spring. It is not clear whether the infundibular receptacles or the vaginal sperm storage areas of snakes are involved in the extremely long cases of delayed fertilization (Haines, 1940; Mangusson, 1979). Evidence derived from the garter snake, where the infundibular receptacles are evacuated upon mating, would suggest that prolonged storage might involve the vaginal receptacles. On the other hand, the superficial location of vaginal sperm storage areas argue against this location for prolonged storage.

#### Albumen receptacles

In contrast to the vaginal and infundibular receptacles of squamate reptiles, sperm-storing tubules of the turtle oviduct are located approximately midway between the cloaca and the ovary. They are found within the lumen of tubular glands in the caudal centimeter of the albumen-secreting segment of the oviduct (Figure 4). These tubules represent the caudal segments of the extensive albumen-secreting portion of the tuba. Immediately posterior to the region of sperm-storing tubules is the transition to the uterus.

The tubular glands containing sperm occupy the lamina propria and are less complex than those in the mid-portion of the albumen-secreting region. The cells comprising the tubules, however, appear identical to those which form the more extensive albumen-secreting tubules. The tubules are formed from 5-6 wedge-shaped cells with basal nuclei and are filled with

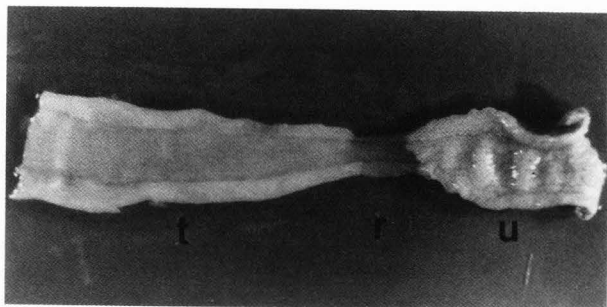


Figure 4. Mucosal epithelium of the oviduct of the painted turtle *C. picta*. Legend as in Figure 1. Actual size.

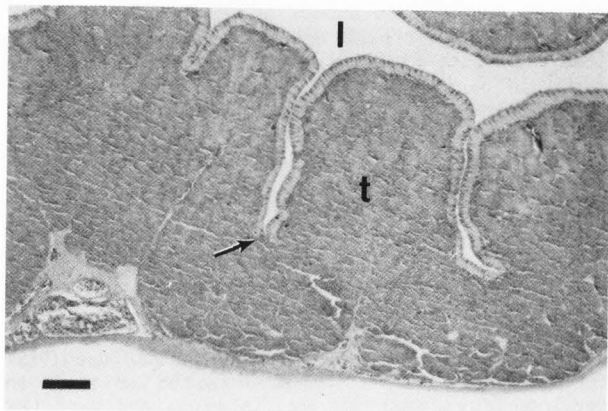


Figure 5. Light micrograph of a cross section through the tuba region of the oviduct of the slider *Pseudemys scripta*. Note longitudinal folds in the oviduct wall separated by thin grooves. The lamina propria is filled with tubular albumen glands (t). l. lumen of oviduct. Arrow: opening of tubular glands at base of groove. Bar = 0.1 mm. Paraffin embedded; hematoxylin and eosin.

eosinophilic granules. They are devoid of cilia and other surface modifications.

Aitken and Solomon (1976) have studied the ultrastructure of the albumen-secreting cells in the green sea turtle *Chelonia mydas*. These cells possess numerous membrane-bound granules of varying electron densities. Prior to ovulation, there may be an inconspicuous Golgi complex on the apical side of the nucleus and large quantities of rough endoplasmic reticulum and electron-dense granules. Following ovulation, the albumen-secreting cells display some degranulation, but many still remain and these granules are principally of the dielectronic variety. These same granules may also be found extracellularly in the lumen of both the tubules as well as that of the oviduct itself. Solomon and Baird (1979) found sperm "nests" at the

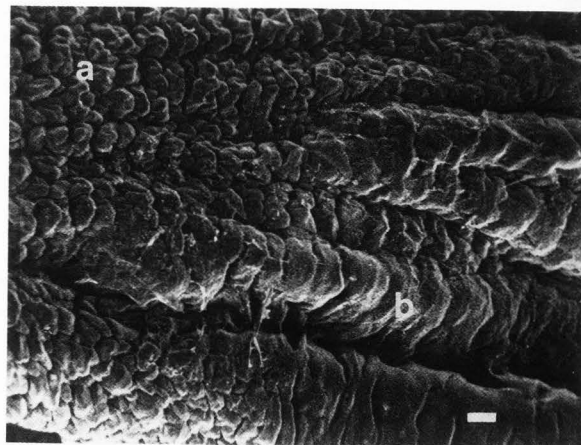


Figure 6. Scanning micrograph of the luminal surface epithelium of the oviduct of *C. picta* at the junction of the tuba and transition regions. a. Mucosal folds of tuba overlying albumen glands; b. mucosal folds of the transition region. Bar = 100µm. Tissues were fixed in 2% glutaraldehyde buffered with 0.1 M cacodylate. Samples were dehydrated, critical-point dried from CO<sub>2</sub>, and sputter-coated with gold palladium. Specimens were viewed using an ISI Mini-SEM.

junction of what they term the magnum and the aglandular zone. This is the same portion of the oviduct that Hattan and Gist (1975) found stored sperm in the box turtle. Solomon and Baird (1979) did not describe the structure of these sperm nests, but did remark that they represented the normal glands of the area.

Tubules of the albumen glands communicate with the oviduct lumen in several ways. Throughout most of the tuba, short ducts are formed from depressions in the epithelium lining the oviduct. These ducts are most common in the grooves separating the folds, and less frequent on the apices of the folds themselves (Figure 5). Giersberg (1923) and Hattan and Gist (1975) found evidence of secretion reaching the lumen through breaks in the epithelium. Breaks were observed consistently in the midportion of the tuba and could occur anywhere on or between the mucosal folds. In the area of sperm storage, mucosal folds are narrower (Figure 6) and the ducts connecting the tubular glands with the lumen more numerous. Often, several ducts may open at the base of a single groove (Figure 7). In this region of the oviduct, the grooves between the mucosal folds appear deeper and wider, probably due to the diminished number of tubular glands in the lamina propria.

Sperm contained within the tubules are positioned with their heads oriented towards the terminal portions of the tubule. The sperm stored in the albumen tubules appear normal. Sperm obtained from scrapings of the oviduct wall of *Chrysemys picta* isolated from males for 14 months were similar to those recovered from oviduct flushes of recently inseminated turtles

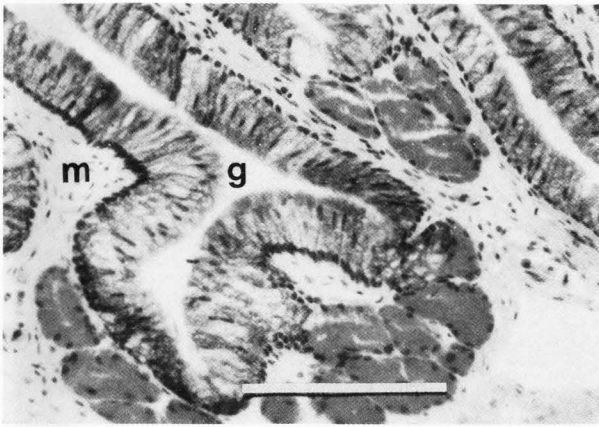


Figure 7. Light micrograph of tubular albumen glands at the tuba-transition junction of the oviduct of *C. picta*. Note ducts at base of groove (g) between mucosal folds (m) leading to secretory tubules. Paraffin embedded; hematoxylin and eosin. Bar = 1mm.

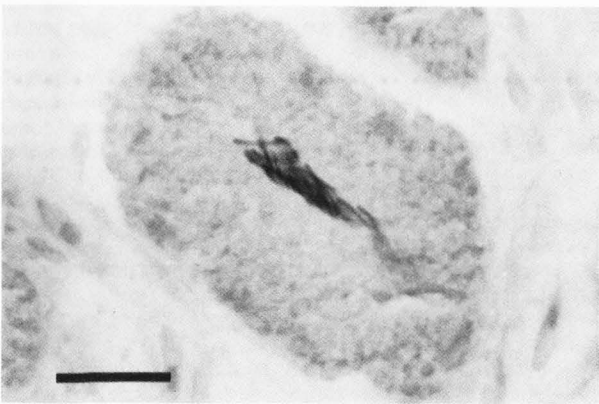


Figure 9. Light micrograph of a tubular albumen gland from the posterior tuba of the oviduct of *C. picta*. Note coiling pattern of sperm stored within the tubule. Bar = 10  $\mu$ m. Paraffin embedded; hematoxylin and eosin.

(Figure 8).

When present in large quantities, sperm frequently are twisted and coiled together and appear as whorls (Figure 9). Unlike the infundibular receptacles of lizards and snakes, there does not appear to be a close association between stored sperm and cells of the tubules comprising the luminal wall. The lumen of tubules containing sperm is frequently enlarged, and the sperm masses remain separate from the tubule wall (Figure 10). The fine structure of the sperm-containing tubules remains uninvestigated, however, and it is possible that the separation between the sperm and the tubule wall may be a fixation artifact.

Quantities of sperm stored within the

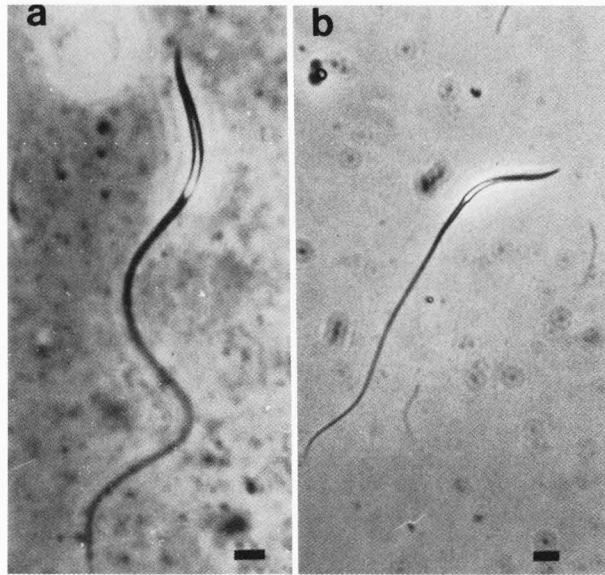


Figure 8. Light micrograph of sperm recovered from oviduct of the turtle *C. picta*. a) stored sperm cell obtained by scraping the mucosal lining of the posterior tuba of a turtle isolated from males for 14 months; b) sperm cell recovered from a saline flush of the oviduct of a recently inseminated turtle. Phase optics. Bar = 1  $\mu$ m.

albumen secreting tubules vary considerably. Hattan and Gist (1975) observed aggregations of sperm "ranging from several to several hundred" in tubules from the box turtle. Of nine species examined by us, the box turtle (*T. carolina*) possessed the largest quantity of stored sperm, while the spiny soft-shelled turtle (*Trionyx spiniferous*) contained the least. We have also examined histologically the oviducts of over 20 painted turtles (*C. picta*) collected from various sites. Females sampled from the same population and from different populations were all quite variable in the amount of stored sperm, although each adult female examined by us did, in fact, contain stored sperm. However, the prior reproductive history of none of these turtles was known. Thus factors such as length of time since the last mating, frequency of mating, and the age of the turtles, which undoubtedly influence the number of stored sperm, are unknown.

We hypothesize that sperm enter the storage tubules via the ducts opening at the base of the mucosal folds. Grooves between the mucosal folds of the posterior portion of the albumen region appear to attract sperm in freshly inseminated animals. Hattan and Gist (1975) suggested that sperm were attracted towards the ducts at the base of the mucosal folds in this region. We have observed similar aggregations of sperm between mucosal folds in recently inseminated *C. picta* (Figure 11). In favorable histological sections, sperm appear to be entering the glands via the epithelial ducts. The posterior portion of the



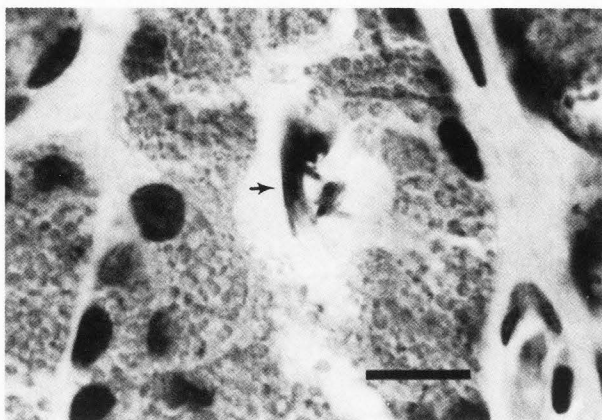


Figure 10. Light micrograph of sperm stored in the tubules of the posterior tuba of the turtle *C. picta*. Note enlarged lumen and apparent lack of interaction between sperm (arrow) and tubule wall. Bar = 1 $\mu$ m. Paraffin embedded; oil immersion; hematoxylin and eosin.

tuba appears to be particularly attractive to sperm occupying the oviduct lumen. To determine the location of luminal sperm within the oviduct, segments were flushed and the perfusate examined for the presence of sperm. In flushes of different regions of the oviduct of 16 turtles collected between May and September, sperm were found only at the level of the posterior albumen glands and below. Aggregations of sperm in the vicinity of ducts leading to storage tubules as well as the absence of sperm from the oviduct lumen anterior to the storage tubules suggest that there is an attraction between sperm and the posterior albumen region. What this attraction might be is unknown.

A possible mechanism for concentrating sperm in the posterior tuba is suggested by the studies of Parker (1931). He examined the movement of sperm up the avian and testudine oviduct, and found that cilia lining the lumen of the albumen region maintained movement both towards the ovary and away from it. The majority of the region was covered by abovarian cilia, but a narrow band running the length of the tuba maintained a proovarian movement. He suggested that sperm movement through the tuba was passive, and that the narrow band of proovarian cilia was instrumental in transporting sperm towards the ovary. If so, the abovarian cilia could prevent sperm from entering the mid tuba region of the oviduct, thus concentrating them in the posterior tuba and transition areas.

How stored sperm move from the tubules in the lower albumen region to accomplish delayed fertilization is not clear. Sperm storage tubules are not surrounded by smooth muscle or myoepithelial cells. Since the storage tubules are morphologically similar to those of the albumen-secreting tubules in the main body of the tuba, their evacuation would appear to be associated with the secretion of albumen. In the

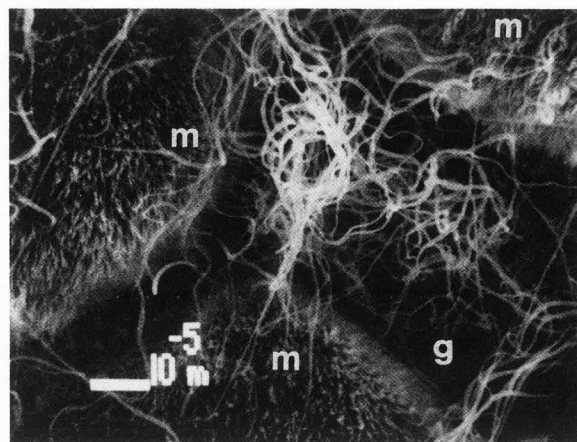


Figure 11. Scanning micrograph of the mucosal folds (m) and grooves (g) from the posterior tuba of the oviduct of *C. picta*. Turtle was recently inseminated and contained luminal sperm in all regions of the oviduct posterior to the tuba. Note sperm aggregated above groove. Bar = 10  $\mu$ m.

hen, secretion of albumen and other egg white constituents from the magnum is thought to occur mechanically as the ovum stretches the oviductal wall and forces secretory material to the lumen (Aitken, 1971; Solomon, 1983). The ovum of the hen does move more rapidly through the magnum than other regions of the oviduct (Aitken, 1971). It has been estimated that the magnum of the hen oviduct at any one time contains sufficient material for two eggs (Solomon, 1983). One might anticipate that the albumen content of the turtle oviduct is considerably greater. For example, green sea turtles ovulate in excess of 100 follicles; once ovulation is initiated, it proceeds rapidly until all follicles of ovulatory size have ruptured and passed to the oviduct (Solomon and Baird, 1979); they also lay multiple egg clutches within a short period of time (Licht et al 1979). While other turtles do not have such large or frequent egg clutches, the albumen which is released from the oviduct must be rationed so as to provide adequate amounts for all eggs ovulated at a given time. Both Giersberg (1923) and Hattan and Gist (1975) noted that albumen was released via breaks in the epithelium as well as through the ducts between the mucosal folds. Available evidence, although sparse and indirect, favors a mechanical releasing of the albumen from the turtle oviduct. If so, it is possible sperm may be delivered to the lumen via the numerous ducts in the posterior albumen region as albumen is forced from these same ducts by the passage of eggs. Sperm, like albumen, seem to be rationed for future ovulations. Hattan and Gist (1975) observed stored sperm in box turtle oviducts containing shelled eggs *in utero*. They suggested that this sperm could be used for future fertilizations.

Significance of sperm storage

Whether stored sperm are capable of successful fertilizations is not known for certain, although the numerous reports of delayed fertilization would argue that they are. Mechanical scrapings of the wall of the oviduct of *C. picta* yield stored sperm which are morphologically intact (Figure 8) and a small percentage of which are motile. Thus at least some stored sperm possess characteristics required for successful fertilization. On the other hand, studies on the garter snake (Whittier and Crews, 1986) indicate that multiple matings are common. Evidence for multiple paternity also exists for this species (Blanchard and Blanchard, 1941). These would suggest that residual sperm in the oviduct as well as newly inseminated sperm both are capable of fertilization. Given the observations of mating behavior in some turtle species throughout the year (Moll, 1979), one might anticipate that turtles also engage in multiple matings. It has yet to be established, however, whether mating at different times of the year each result in sperm transfer. Copulations with or without ejaculations are known to have a positive influence on mammalian fertility, and are suspected to influence reptilian fertility as well (Bona-Gallo and Licht, 1983).

Sperm storage is particularly useful to those oviparous vertebrates possessing multi-egg clutches. Given the highly telolecithal egg of reptiles, it is likely that sperm occupying the oviductal lumen would be forcefully evacuated from the oviduct as an egg passed through the upper oviduct towards the uterus for shell deposition. If more than a single egg were shed in a given ovulatory period, then the female must either store sperm or remate. Given the energetic and social costs of continued matings, it is clearly more efficient for the female to store sperm for subsequent ovulations. It is perhaps significant that sperm storage within the oviduct is a reproductive strategy employed by avian (Bakst, 1987) as well as reptilian species.

Storage of sperm within the oviducts of turtles presents an added dimension to the already paradoxical reproductive cycle of turtles. It is well known that in temperate species, spermatogenesis and oogenesis occur at separate times of the year (Moll, 1979; Crews, 1984) and are not necessarily correlated with annual endocrine cycles (Licht, 1982). It is generally thought that temperate zone turtles mate in the spring following emergence. This is a time of spermatogenic arrest and low levels of male sex hormones. Spermatogenesis is completed in autumn, and it is generally thought that sperm overwinter in the epididymis. However, there are many reports of fall copulations in temperate turtles, and the existence of sperm storage areas in female turtles suggests that these fall copulations provide a reservoir of sperm to be used for fertilization the following spring. The longevity of sperm stored within the albumen glands of turtles attests to the favorable environment provided by the tubules, but fertility does decline with time (Hildebrand,

1929), presumably as the result of sperm depletion. Thus, the prolonged instances of delayed fertilization may not reflect the normal reproductive process. Whether sperm storage is a mandatory component of the annual reproductive cycle of turtles, or simply an alternative, must await final determination of the precise time of insemination.

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#### Discussion with Reviewers

B. R. Howarth Jr.: The authors present an interesting hypothesis concerning the release of spermatozoa from the tubules in the lower albumen secreting region of the tuba in the turtle (*C. picta*). They postulate that sperm evacuation might be linked to the secretion of albumen thought to occur mechanically as the ovum stretches the oviductal wall in passing through this region of the reproductive tract. A similar hypothesis has been suggested for the evacuation of spermatozoa from the utero-vaginal sperm host glands of the hen as a shelled egg passes through that region of the oviduct during oviposition. In the hen, however, ovulation of the next ovum seems to be synchronized with oviposition occurring some 20 to 40 minutes later. Thus, sperm released at the time of oviposition have 20 - 40 minutes for transport through a relatively empty oviduct to the infundibulum where fertilization of the next ovulated ovum would occur. This time period has been demonstrated to be sufficient for chicken and turkey spermatozoa to reach the site of fertilization.

I have two questions with respect to the feasibility of this hypothesis as it pertains to the release of turtle spermatozoa:

1. What effect would the coincident release of sperm and albumen have on sperm transport to the site of fertilization?

2. What effect would the acquisition of additional investments (i.e. albumen) have on the fertilization of the turtle ovum?

I raise these questions with respect to our current knowledge of the fertilization process in the hen. The fertilizable life span of the hen's ovum after ovulation is estimated to be 15 - 20 minutes or about the time the ovum spends in the infundibulum. Upon entering the distal infundibulum and proximal magnum, the ovum acquires additional investments which block sperm penetration. Several factors, including differences in amino acid composition of these additional investments and the presence of trypsin inhibitors have been discussed as

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possible reasons for failure of cock spermatozoa to penetrate these tertiary membranes.

Authors: The site of fertilization in the turtle, as in the hen, is believed to be the infundibulum. Sperm released from storage tubules in the turtle would have to move through the tuba, a distance shorter than in the hen where sperm must move across both the uterine and magnum areas of the oviduct to the infundibulum. Sperm coincidentally released from the glands with secreted albumen might remain in the ducts in between the folds until an egg has passed to the uterus before resuming their movement towards the infundibulum. These ducts, which connect the albumen glands with the oviduct lumen, are most common in the storage portion of the tuba. Whether this mechanism is feasible depends upon the time between ovulations. The time interval between the ovulations in a multi-egg clutch, such as occurs in turtles, is not known, but may be of sufficient duration to allow the necessary sperm movements to occur.

With respect to the second question, if the turtle ovum were surrounded by albumen at the time of fertilization, then fertilization would have to occur at the level of the tuba or lower. Turtle albumen is less viscous than its avian counterpart, and would provide less of a physical barrier for sperm penetration. Biochemical factors which might influence sperm penetration of turtle membranes are unknown.