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ANATOMICAL BASIS OF SPERM-STORAGE IN THE AVIAN OVIDUCT

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Abstract

The anatomical basis of oviductal sperm-storage in the avian oviduct is the sperm-storage tubule (SST). In the turkey this is composed of a simple columnar epithelium characterized by a supranuclear vacuole, which possibly contains lipid material, and appears to differentiate from the oviductal surface epithelium prior to full sexual maturation. Turkey SST epithelium was periodic-acid Schiff-negative and failed to bind four of the five lectins examined. Only winged pea agglutinin, which has an affinity for fucose-containing glycosidic residues, bound to some individual SST epithelial cells. Short-term cultures of oviductal mucosae containing SST showed aggregates of sperm associated with the spaces between adjacent folds and in surface openings assumed to be SST.

KEY WORDS: Turkey, Bird, Fertility, Semen Preservation, Sperm-Storage Glands, Oviduct, Sperm.

Introduction

The capacity for prolonged sperm-storage in birds obviates the need for the synchronization of copulation and ovulation by the female during the so-called "fertile-period" (Lake, 1975). Although questions remain as to whether all female birds have the capacity or need to store sperm (Astheimer, 1985), oviductal sperm-storage appears to be of wide taxonomic occurrence. Based on histological examinations of oviductal tissue from specimens representing seven taxonomic families (reviewed by Hatch, 1983; Bakst and Bird, 1987), the anatomical basis for sperm-storage is the sperm-storage tubule (SST) located at the uterovaginal junction.

In most birds, only the left Mullerian duct develops into a functional oviduct. Anatomically and functionally the oviduct is divided into five distinct segments [see Gilbert, (1979) for a detailed discussion]. The infundibulum, which is the most anterior segment, possesses the fimbria used in grasping and guiding the ovulated ovum to the ostium of the oviduct. It is also the site of fertilization. Just distal to the infundibulum is the longest and widest segment of the oviduct, the magnum. Its surface epithelium and subepithelial tubular glands synthesize and secrete the proteins comprising the egg albumen. A narrow translucent band in the oviductal mucosa separates the magnum from the more distal isthmus. The isthmus, which is shorter and narrower than the magnum, forms a fibrous reticulum of connective tissue, the shell membrane, around the egg mass. The shell membrane provides the seeding sites on which the calciferous shell is formed. Shell deposition is the primary function of the uterus (also referred to as the shell-gland), which unlike the other segments, has a pouch-like appearance. The most posterior segment is the vagina which serves as a conduit between the uterus and the cloaca. When visualized grossly, the vagina appears as a thickened mass of connective tissue extending from the uterus. Though not considered a segment, the uterovaginal junction is significant as here are located the primary sperm-storage sites in the bird oviduct, the SST.

Attempts to define the mechanisms of sperm survival within the SST of the chicken and turkey have been limited primarily to relating SST histology, histochemistry, and ultrastructure with the known fertility of the hen. At present no correlates have been established between high or low hen fertility and SST morphology (Schuppin et al., 1984). In the

present study, the literature regarding oviductal sperm-storage in birds is reviewed and augmented with new, preliminary observations from this laboratory on the histology and lectin histochemistry of the turkey uterovaginal junction and SST epithelia. Lectins, which are glycoproteins of plant origin with affinities to specific carbohydrates, have been used to identify and localize specific carbohydrate residues in tissue sections not identifiable by standard histological procedures.

Materials and Methods

Turkey (Meleagris gallopavo) hens were caged individually in an environmentally-controlled house at 28 or 30 weeks of age and given free access to feed and water. Hens were exposed to stimulatory light (photoperiod of 14 h light and 10 h dark) within one week of being caged and came into egg production 2 to 3 weeks later. Unless stated otherwise, hens were inseminated on Days 12 and 13 after the onset of photostimulation and weekly thereafter with 100 million sperm. Eggs were collected daily, stored in a cool-room, and once a week set in an incubator. Fertility was determined by candling at 7 days of incubation.

Oviductal tissues were collected from hens euthanized with sodium pentobarbital. A detailed explanation for the localization and isolation of the uterovaginal junction mucosa containing SST is provided in the Results and Discussion section. All exposed mucosal surfaces were kept moist with Hank's Balanced Salt Solution (Hanks) until the desired tissue specimens were isolated and fixed. For light microscopy, specimens were fixed in either neutral buffered formalin or Carnoy's and processed by standard procedures for paraffin embedment. Four to five micrometer thick sections were stained with periodic acid-Schiff, or with either horseradish-peroxidase or fluorochrome conjugated lectins. The taxonomic and common names, carbohydrate specificity, and inhibitory sugar of each lectin used in this study are found in Table 1.

For lectin staining (lectins obtained from Sigma, St. Louis, MO), only tissues from hens with fertility greater than 85% and 10 to 11 weeks into egg production were examined. All sections were carried through the following steps at ambient temperature:

1) Sections attached to slides coated with Histostik (Accurate Chemical & Scientific Corp., Westbury, NY) were deparaffinized in two 5 min changes of xylene and air-dried;

2) Sections were rehydrated in two 5 min changes of 0.05 M TRIS HCl (pH 7.5 at 25 C) plus 0.1 mM of calcium, magnesium, and manganese chlorides (buffer). Sections stained with horseradish peroxidase-conjugates were then incubated for 5 min with 3% hydrogen peroxide (prepared fresh) and the buffer wash repeated as described above;

3) Sections of neutral buffered formalin fixed tissue were flooded with protease (1 mg/ml buffer) (Brandtzaeg, 1981) for 15 min followed by three 3 min buffer washes;

4) Sections were flooded with lectin (100 micrograms/ml buffer for horseradish peroxidase-conjugates and 10 micrograms/ml buffer for fluorochrome-conjugates) for 2 h followed by three 3 min buffer washes;

5) Sections stained with the fluorochrome-conjugates were mounted with coverslips and viewed with an epi-illuminated fluorescence light microscope while sections stained with horseradish peroxidase-conjugated lectins were incubated in 0.05% diaminobenzidine in buffer for 15 min before and for 20 min after the addition of hydrogen peroxide (final concentration 0.005%). Sections were washed in buffer and stained lightly with hematoxylin before viewing. Control sections were prepared by either omitting the lectin or by coupling the lectins with 0.2 to 0.4 M of appropriate inhibitory sugars (Table 1) before lectin staining.

For scanning electron microscopy, excised mucosal folds containing SST were placed in a fixative of 2% formaldehyde and 1% glutaraldehyde in 0.15 M cacodylate buffer (pH 7.2) and refrigerated for a minimum of two days. Trimmed specimens were fixed again in 1% osmium tetroxide in 0.1 M cacodylate buffer, dehydrated in ethanol, and critical-point dried. Specimens were viewed with a Hitachi S-430 microscope operated at 15 kV.

Uterovaginal junction explants were maintained in vitro using a technique adapted from Nash et al. (1986). Tissues were removed from hens as described above and placed in Hank's instead of fixative. Each piece of tissue was glued to a single layer of dialysis membrane (10,000 molecular cutoff) and the dialysis membrane was positioned tissue side down across the lower chamber of a separation cell containing 1 ml of $1x10^9$ sperm in Hanks. The upper chamber was locked in place and moist air was directed through the otherwise closed system. After 90 min at room temperature the dialysis membrane containing the explant was rinsed briefly in Hanks, placed in fixative, and prepared for scanning electron microscopy as described previously.

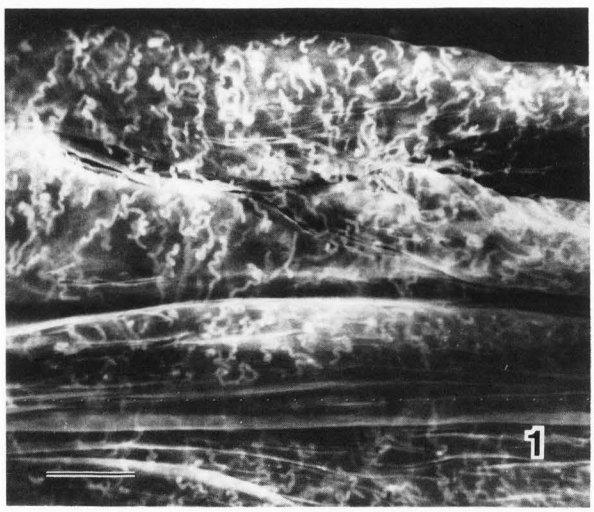
Results and Discussion

Located at the uterovaginal junction are discrete nonbranching tubular invaginations of the surface epithelium, here referred to collectively as the SST (Figs. 1 and 2). (Although more frequently referred to as sperm-storage glands, the SST are not true glands and are thought to not be capable of extensive secretory activity). Methodology for the precise localization of the SST, which has been successfully adapted to diverse species ranging in size from the American kestrel (Bakst and Bird, 1987) to the rhea (unpublished, Bakst), consists of isolating the uterus and vagina, carefully removing the connective tissue surrounding the distal region of the uterus and vagina and making a longitudinal cut through both segments. Individual mucosal folds are isolated starting at the distal end of the uterus and continuing to about half the length of the vagina. The presence of SST within each isolated fold was confirmed by stereomicroscopy using a light source directed at the specimen in a path parallel to the microscope base (Goodrich-Smith and Marquez, 1977). When viewed in this manner individual SST are highlighted brightly against a dark background. Another method which has been used to isolate SST from the chicken and turkey involves the inversion of the vaginal mucosa up to the uterovaginal junction. Here three distinct circular rings are observed, the most anterior of which contains the SST (Ogasawara and Marquez, 1974).

Table 1. Lectins used for histochemical staining

Lectin/Agglutinin (A)		
Taxonomic Name	Common Name	Sugar specificity
Canavalia ensiformis[1]	Concanavalin A (Con A)	alpha-D-Mannose alpha-D-Glucose
Triticum vulgaris[2]	Wheat germ (WGA)	beta-N-Acetylglucosamine N-Acetylneuraminic acid
Arachis hypogaea[3]	Peanut (PNA)	beta-D-Galactose
Glycine Max[3]	Soybean (SBA)	N-Acetylgalactosamine alpha-D-Galactose
Tetragonolobus purpureas[3]	Winged pea (WPA)	alpha-L-Fucose

1 Fluorescein isothiocyanate conjugated; 2 Tetramethylrhodamine isothiocyanate conjugated
3 Horseradish peroxidase conjugated



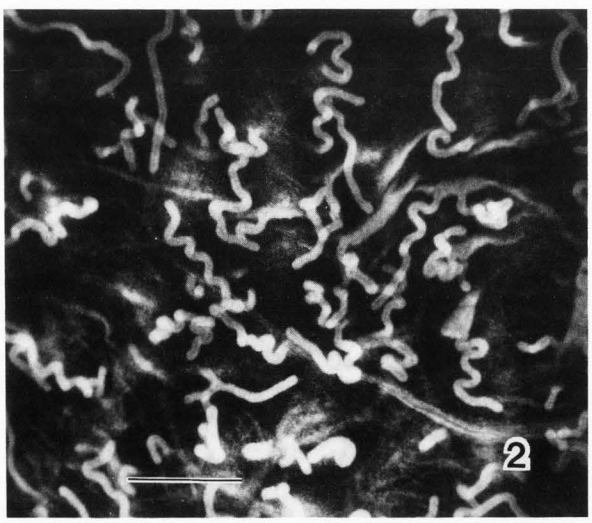


Figure 1. A photomicrograph of a fresh piece of UVJ tissue containing numerous sperm-storage tubules within the mucosal folds. Bar = 1,000 micrometers.

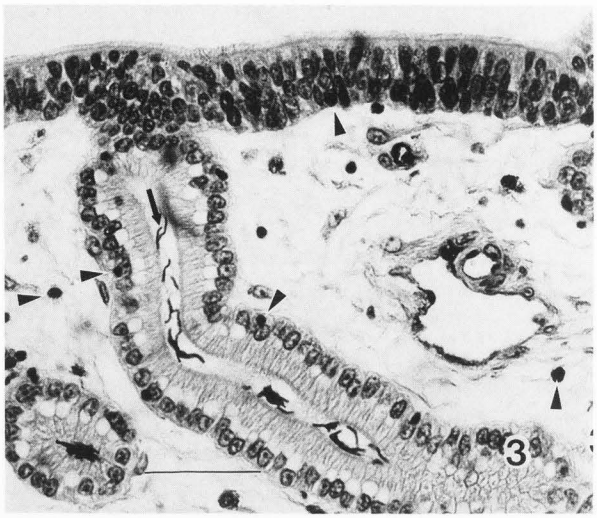
Figure 2. A higher magnification of the same tissue preparation observed in Fig. 1. Discrete non-branching sperm-storage tubules of various configurations are evident. Bar = 500 micrometers.

Differentiation of the turkey SST appears to be under the control of estrogen and/or progesterone (Pescatore and Marquez, 1977) and begins prior to the onset of laying (egg production usually begins 2 to 3 weeks after the onset of photostimulation) and continues through at least the first 8 weeks after the onset of photostimulation (Goodrich-Smith and Marquez, 1978). Recent observations (unpublished, Bakst) show the presence of SST in 30 week old hens not exposed previously to stimulatory light and possessing an undeveloped ovary and oviduct.

The pseudostratified surface epithelium lining the uterovaginal junction consists of ciliated and non-ciliated columnar cells (Fig. 3). Generally, the apical half of the ciliated cell contains the nucleus and consequently is wider than its more attenuated basal half. The non-ciliated cell is more variable in shape and the nucleus is located in its basal half. The turkey uterovaginal junction-surface epithelium is characterized by alternating periodic-acid Schiff positive and negative staining cells (Fig. 4). The surface epithelium stained more uniformly positive with periodic-acid Schiff in the deeper mucosal folds than near the lumen.

In contrast to the surface epithelium, the SST consists of a simple columnar, non-secretory epithelium (Figs. 3-7). Characteristically, nuclei are in the basal third of the SST epithelial cells and a large supranuclear vacuole, possibly an artifact resulting from the extraction of lipid during tissue preparation, is often present. The transition between uterovaginal junction and SST epithelia is abrupt and easily discernible (Figs. 4 and 5, Table 2).

Randomly dispersed in the surface epithelium of hens 32 to 36 weeks of age (around the onset of egg



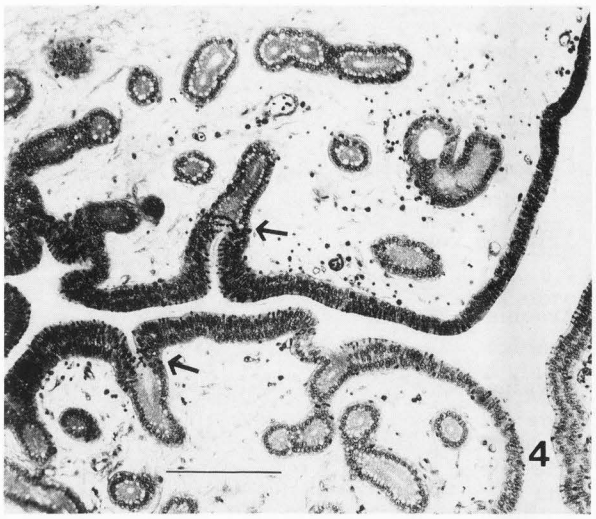


Figure 3. A light micrograph showing the pseudostratified ciliated columnar epithelium of the uterovaginal junction and the simple columnar epithelium of the sperm-storage tubule (SST), the latter of which is characterized by the presence of supranuclear vacuoles. Dense staining cells in the epithelia (arrowheads) and loose connective tissue are soybean agglutinin-positive lymphocytes. The elongated sperm in the SST (arrow) and other cell nuclei are stained with hematoxylin. Bar = 36 micrometers.

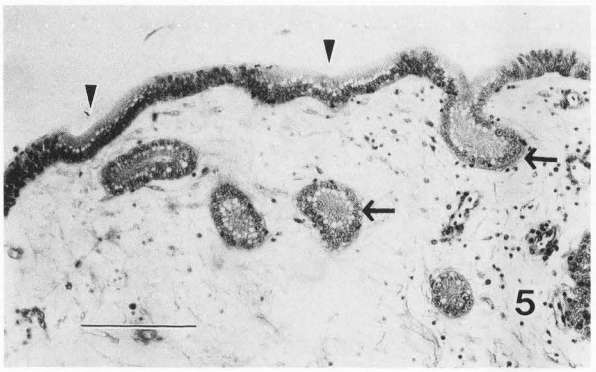
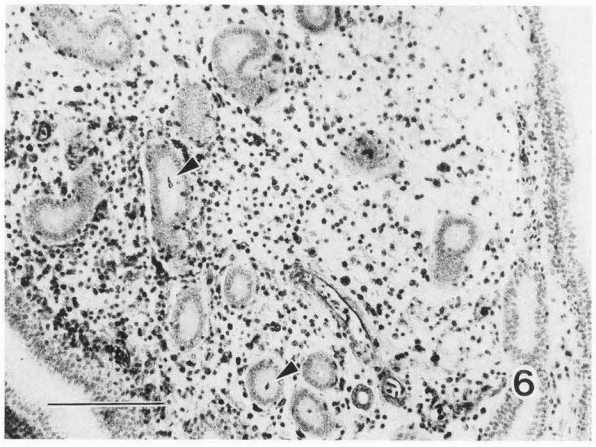


Figure 4. A survey micrograph showing the transition between the uterovaginal junction surface epithelium with alternating PAS-positive and negative cells and the PAS-negative sperm-storage tubule epithelium. Arrows indicate points of transition. Bar = 91 micrometers.



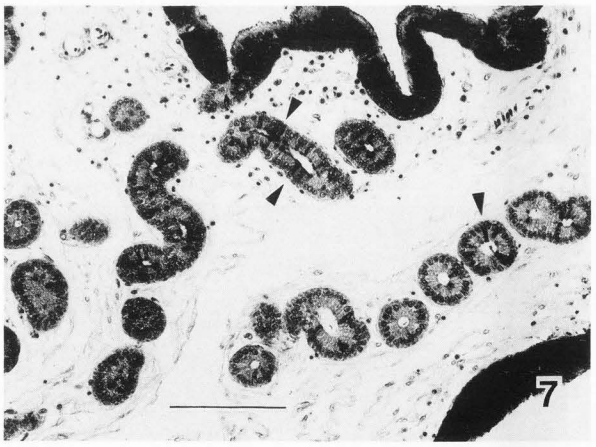


Figure 5. Two groups of contiguous PAS-negative epithelial cells (arrowheads) are observed in the uterovaginal junction surface epithelium. These contiguous cells resemble cells comprising the sperm-storage tubule epthelium (arrows). Bar = 91 micrometers.

Figure 6. Lymphocytes in the loose connective tissue of the uterovaginal junction bind densely peanut agglutinin (PNA), whereas the sperm-storage tubule (SST) epithelium fails to bind PNA. Sperm are observed in a few SST (arrowheads). Bar = 91 micrometers.

Figure 7. Winged pea agglutinin is the only lectin which binds intensely to nearly all uterovaginal junction surface epithelial cells and selected sperm-storage tubule epithelial cells (arrowheads). Bar = 91 micrometers.

production) were groups of 20 to 40 contiguous periodic-acid Schiff negative cells with a supranuclear vacuole (Fig. 5). Cells in such clusters resemble SST epithelium and may represent local sites of epithelial cell differentiation and proliferation prior to actual cell invagination.

Table 2. Comparison between uterovaginal junction and sperm-storage tubule epithelia based on light microscopy of paraffin embedded tissue sections

Uterovaginal junction surface epithelium	Sperm-storage tubule epithelium
Pseudostratified columnar	Simple columnar
Secretory and ciliated cells	Nonciliated, nonsecretory cells
Alternating PAS-positive cells	PAS negative
Con A, WGA, WPA positive	WPA positive
No supranuclear vacuole in most cells	Supranuclear vacuole

Based on lectin binding patterns, glycosidic residues specific for PNA and SBA in the surface and SST epithelia are either sparse or masked by other glycosidic residues. If PNA or SBA binding occurred, it was restricted to a narrow zone in the apical cytoplasm of surface epithelial cells (Figs. 3 and 6). Con A and WGA binding patterns resembled the binding pattern observed after periodic-acid Schiff staining, i.e., SST epithelium failed to bind either lectin, and about 50% of the surface epithelial cells bound Con A and WGA in their apical cytoplasm. Of all the lectins tested WPA bound most extensively throughout the surface epithelium and selectively in the SST epithelium. Reaction product was extensive and dense in the supranuclear cytoplasm of the surface epithelial cells but varied considerably in the SST epithelium (Fig. 7). Here individual SST either remained free of WPA or contained individual cells with reaction product. Additional lectins are presently being used to characterize the oviductal epithelial cells of young hens (less than 37 weeks of age) of known high fertility with old hens (greater than 50 weeks of age) of known high and low fertility.

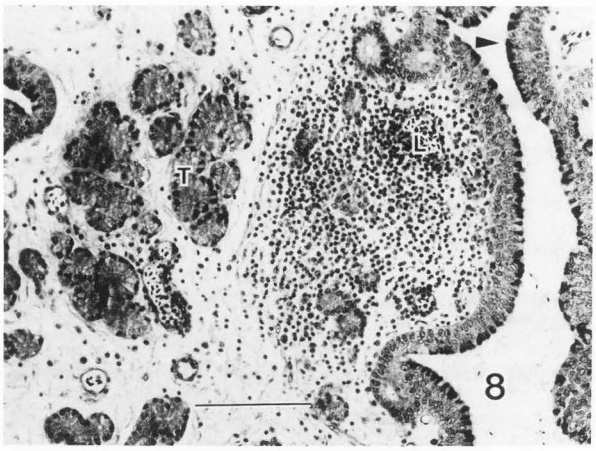
Surface and tubular gland epithelia from the distal infundibulum (included here also is tissue from the infundibulum-magnum junction, which anatomically is a poorly defined region where mucosae from these segments merge), which is considered to be a secondary sperm-storage site in the chicken (Van Drimmelen, 1946; Fujii and Tamura, 1963) and turkey oviduct (Bakst, 1981), exhibited a wide range of affinities for periodic-acid Schiff (Fig. 8), SBA (Figs. 9 and 10) and PNA. Whereas periodic-acid Schiff densely stained alternate surface epithelial cells and less densely the remaining surface epithelial and tubular gland epithelial cells, SBA and PNA failed to bind extensively to the surface epithelial cells except occasionally for a narrow zone in the apical cytoplasm. However, the globular secretory material which occupied most of the volume of the tubular gland epithelial cells bound these two lectins with varying intensities. A transition between infundibular and magnal tubular glands became apparent with the most intense SBA and PNA binding associated with the magnal tubular glands (Fig. 10). A similar transition was observed between the surface epithelial cells and the tubular gland epithelial cells.

Scattered in the lamina propria of the uterovaginal junction and infundibular mucosae were free lymphocytes and within the surface and SST epithelia were intra-epithelial lymphocytes (Figs. 3, 6, 9-12). Schuppin et al. (1984; 1985) observed intra-epithelial lymphocytes in the SST epithelium from both high and low fertility turkeys and suggested that the intra-epithelial lymphocytes may be suppressor T-cells. Some, but not all of the free lymphocytes and intra-epithelial lymphocytes stain densely when incubated with peroxidase-conjugated goat anti-turkey IgG (Fig. 12), SBA and PNA (Fig. 6). Dense aggregates of lymphocytes were observed in the loose connective tissue (Figs. 6, 8, and 9) associated with mucosal capillaries or more rarely with the surface or SST epithelia. Even less frequently were lymphocytes observed in the lumina of SST (Fig. 11). Plasma cells were also observed frequently in the lamina propria. In addition, Schuppin et al. (1985) noted the presence of plasma cells in the epithelium of the initial portion of the SST and suggested that the plasma cells may adversely affect sperm-storage and subsequent fertility.

Also seen in the surface epithelium at this time were round mitotic figures. Whether these cells were proliferating columnar epithelial cells or lymphocytes could not be determined.

In the Galliformes examined, including the chicken (Fujii, 1963; Fujii and Tamura, 1963; Bobr et al., 1964; Schindler et al., 1967; Van Krey et al., 1967; Burke et al., 1972; Tingari and Lake, 1973; Fujii, 1975), turkey (Verma and Cherms, 1965; Bradley, 1982; and Schuppin et al., 1984), and Japanese quail (Friess et al., 1978), the SST epithelium is simple columnar and is characterized by a basally located nucleus and perinuclear lipid droplets, the latter visible by light microscopy. As reported in this study, following periodic-acid Schiff and lectin histochemistry, the SST epithelium is essentially negative except for WPA. Histochemical tests for glycogen were negative (Bakst, unpublished) which supports ultrastructural observations by Bradley (1982) and Schuppin et al. (1984). The latter noted only small amounts of glycogen. Similar observations were reported with the domestic duck (Pal, 1977). In contrast to the turkey, histochemical tests of the chicken SST epithelium were positive for glycogen (Gilbert et al., 1968). Transmission electron microscopy confirmed the presence of dense accumulations of glycogen in the chicken SST epithelium (Tingari and Lake, 1973). In one of the few non-domestiated species examined, the SST epithelium of the American kestrel exhibited some periodic-acid Schiff-positive granules in the apical portion of the cells (Bakst and Bird, 1987).

A common feature of the SST epithelial cells in the Galliformes examined is the perinuclear lipid accumulations. However, the precise composition of the lipid remains unknown. Using histochemical procedures with chicken tissue, Fujii (1963) identified cholesterol and no phospholipids in the SST while



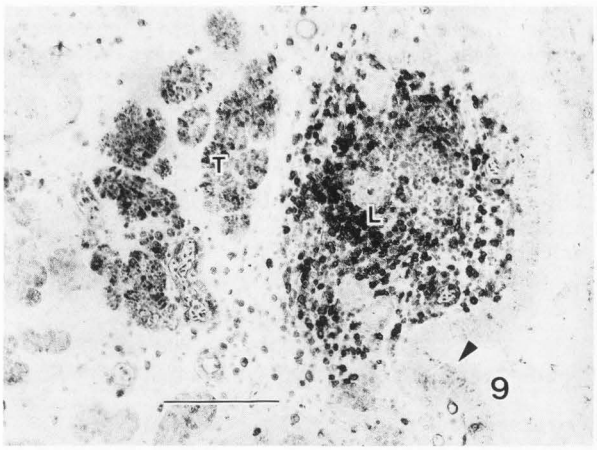
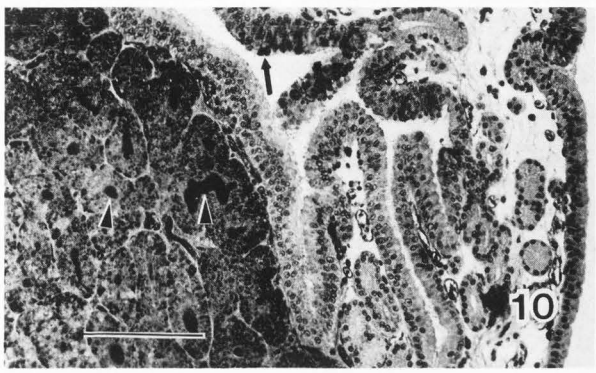
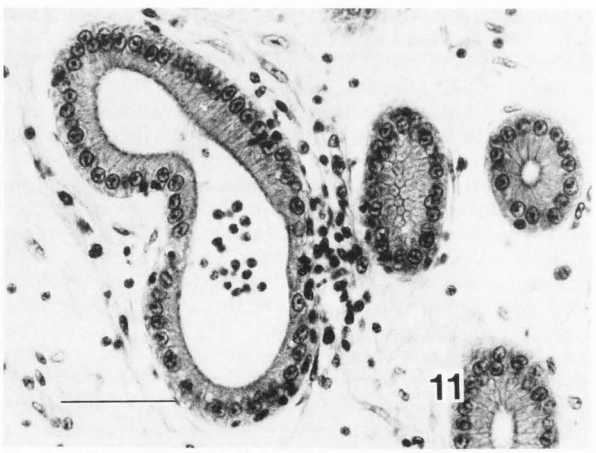


Figure 8. Alternating cells in the distal infundibulum surface epithelium stain densely with PAS (arrowhead). In contrast, tubular glands (T) in the lamina propria stain lightly. A dense aggregate of lymphocytes (L) surround a few tubular glands. Bar = 91 micrometers.
Figure 9. A section taken within 60 micrometers of the section of Fig. 8 shows the distal infundibulum after soybean agglutinin (SBA) binding. Soybean agglutinin binding is heaviest over the lymphocytes found both in the dense aggregate of lymphocytes (L) and elsewhere in the loose connective tissue. The secretory globules in the tubular gland (T) bind variable amounts of SBA. In contrast, the surface epithelium is nearly devoid of SBA except for some punctate binding sites (arrowhead) (compare with Fig. 8). Bar = 91 micrometers.





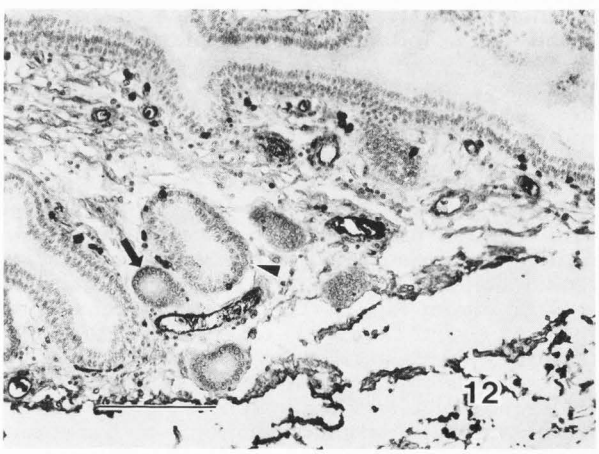


Figure 10. Another section of the distal infundibulum showing soybean agglutinin binding. Here there is an abrupt transition between transitional glands (which fail to bind SBA) and magnal tubular glands (which bind SBA in varying amounts). The lumen of a few tubular glands are distended with SBA-binding material (arrowheads). Few cells in the surface epithelium bind SBA (arrow). Bar = 91 micrometers.
Figure 11. Associated with the epithelium of one sperm-storage tubule (SST) are several lymphocytes. This particular SST is distended and has a few lymphocytes in its lumen. The latter is rarely observed. Bar = 36 micrometers.
Figure 12. Lymphocytes in the loose connective tissue at the uterovaginal junction are positive for turkey IgG. IgG binding is also seen on endothelial walls and deep in the submucosa. A transverse or oblique view of a deep fold or invagination of surface epithelium (see Fig. 13) (arrowhead), which is characterized by a ciliated, pseudostratified columnar epithelium, should be distinguished from a similar view of a sperm-storage tubule, characterized by a simple columnar epithelium (arrow). Bar = 91 micrometers.

Gilbert et al. (1968) observed the opposite. Wall (1975) noted that the histology, including the distribution of lipid, of the turkey SST was similar to that of the chicken. Following extraction of the lipid in the turkey SST, Wall (1975) found cholesterol, cholesterol ester, a fatty acid, and phosphatidyl ethanolamine. Whether one or more of these lipids is utilized by resident sperm is not yet known. The presence of an enzyme (glycerol-1-phosphate dehydrogenase) in the midpiece of quail sperm indicates that sperm residing in the SST may be capable of using lipid-derived phosphotrioses in the glycolytic pathway (Sinowatz et al., 1976). However, it appears that the phosphotrioses would have to be derived from exogenous lipid since chicken sperm stored 24 h either at 41 C (Howarth, 1981) or at 5 C (Resseguie and Hughes, 1984) did not utilize endogenous phospholipids. Such lipids would possibly serve SST sperm as a source of metabolic substrate as well as in the maintenance of membrane integrity.

When viewed by scanning electron microscopy, the openings to the SST are masked by the cilia (Ogasawara and Marquez, 1974; Fujii, 1975). These investigators also noted that the turkey SST are restricted to the first major circumferential fold adjacent to the uterus and that following disruption of the surface epithelium by freeze-drying, grooves in the mucosa are observed associated with and leading to openings to individual SST. It appears that these grooves orient sperm toward the SST. Likewise, in paraffin and plastic sections of uterovaginal junction, openings to the SST are generally located at the base of surface grooves and invaginations lined with periodic-acid Schiff-positive ciliated and non-ciliated cells (Fig. 4). Although the transition from surface SST to epithelium is abrupt and easily discernible based on histological and histochemical characteristics, care must be exercised in identifying transverse sections of such surface invaginations because their size, shape, and distribution resembles that of SST (Fig. 12).

To obtain a better understanding of the mechanisms of sperm acceptance into the SST, a procedure to maintain uterovaginal junction mucosae containing SST (SST explants) in vitro was adapted from Nash et al. (1986). Scanning electron microscopy of the SST explants showed a variable distribution of sperm on the explant's surface. A single explant may have a region devoid of sperm, another region may have sperm dispersed singly or in small aggregates (Fig. 13), yet the surface of another region of the same explant may be masked by a mat of sperm. Sperm in the large "mats" which covered the surface of the explants were not totally randomly oriented. Although not in contact with the surface, sperm heads were oriented parallel to each other and directed perpendicular to the surface of the SST explant (Fig. 14). Aggregates of sperm were most frequently observed in the space between apposed folds (Fig. 13). It could not be determined if such openings led to SST. However, aggregates of sperm were observed partially penetrating round openings, possibly SST, in the surface epithelium (Fig. 15). Further work using

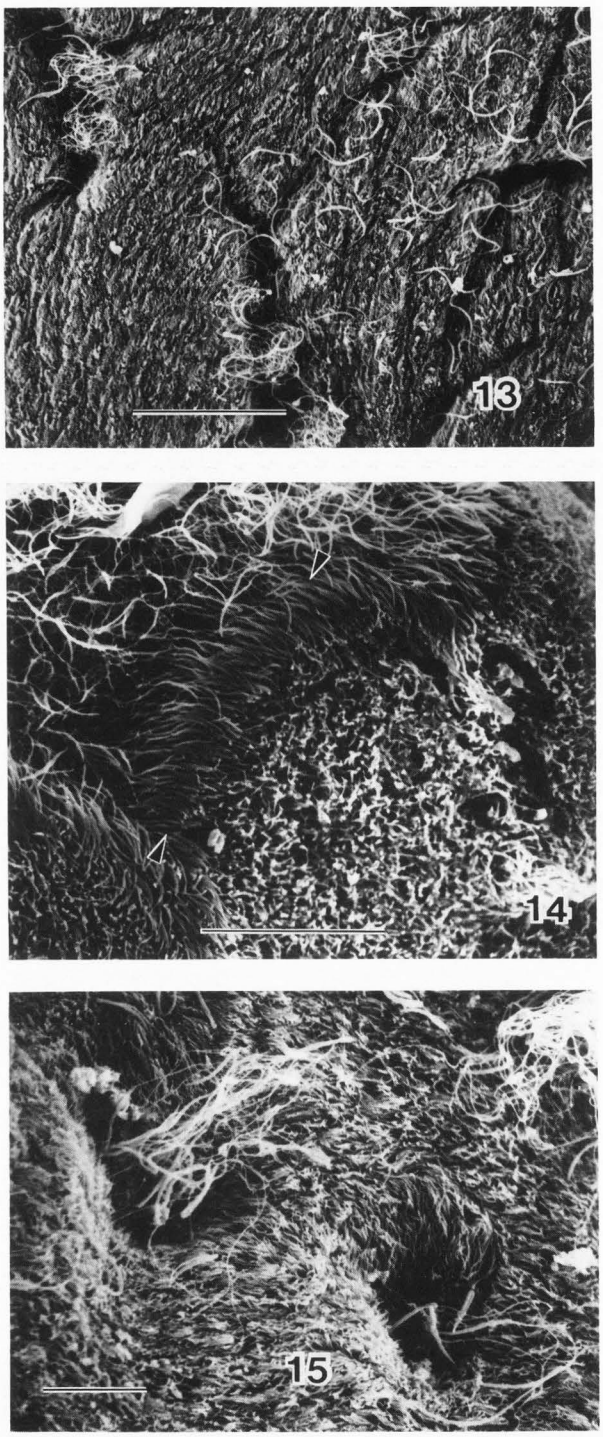


Figure 13. A uterovaginal junction explant after incubation with sperm for 90 min shows individual sperm on its ciliated surface and aggregates of sperm in two of the surface folds. Bar = 50 micrometers.

Figure 14. A dense aggregate of sperm ("mat") which had separated from the UVJ explant's surface reveals a typical parallel-head array (arrowheads) oriented toward the explant's surface. Bar = 50 micrometers.

Figure 15. Sperm are observed oriented head-first into what are believed to be openings of sperm-storage tubules. Bar = 15 micrometers.

the SST explant technique will include the examination of the roles of cilia and/or chemotaxis in sperm acceptance into the SST.

The precise mechanism by which sperm survive within the SST remain unknown. As noted previously (Bakst and Richards, 1985), sperm survival in the SST may involve the reversible suppression of sperm metabolism and motility, stabilization of plasma and acrosomal membranes, inhibition of acrosomal enzymes, and suppression of an immune response to resident sperm. Sperm within the SST are oriented toward the base of the SST and generally exhibit extensive head-to-head agglutination (Van Krey et al., 1981). These authors suggested that such agglutination is a basic mechanism controlling sperm-storage and that the ability of sperm to agglutinate decreases with increasing age of the sperm. Squash preparations of fresh SST-containing-mucosa reveal that most resident sperm are immotile and a few are oscillating slowly (Bakst, unpublished). While earlier investigators suggested that sperm may subsist on oxidizable substrates secreted by the SST epithelium (Fujii, 1963; Van Krey et al., 1967; Gilbert et al., 1968; Freiss et al., 1978), more recent work on the turkey SST ultrastructure suggests that the turkey SST epithelium, lacking the organelles associated with protein synthesis, is not secretory but absorptive (Schuppin et al., 1984). Our most recent work shows that zinc, which has been shown to modulate metabolism and motility in sperm of non-avian species and inhibits lipid peroxidation of membranes, is present in the UVJ mucosa in relatively high concentrations [see Bakst and Richards (1985) for review]. Zinc also depresses turkey sperm oxygen consumption in vitro without affecting fertility (Bakst, 1985).

Conclusion

An understanding of the mechanisms in the oviduct responsible for prolonged maintenance of sperm will augment current efforts in the area of short and long-term storage of sperm in vitro. This would not only be of benefit to the commercial poultry industry but is of great interest to institutions which are presently engaged in both the propagation of endangered avian species and the maintenance of a diverse gene-pool through the cryopreservation of semen.

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Discussion with Reviewers

H.P. Van Krey: Could you interpret and discuss in greater detail the significance of the immunohistochemical results you obtained with the peroxidase conjugated goat anti-turkey IgG?

Author: Goat anti-turkey IgG was used to localize IgG-producing immunocytes in the uterovaginal junctional surface and SST epithelia and loose connective tissue. Many intra-epithelial lymphocytes (IEL) and free lymphocytes and plasma cells in the loose connective tissue failed to bind the antibody indicating that they may produce another immunoglobulin, possibly IgA. The relationship between these immunocytes and the efficacy of sperm-storage in the SST is not known. Dym and Romnell (J. Reprod. Fert. 42, 1-7, 1975) suggested that the IEL in the male mammal reproductive tract sequester sperm antigens thereby preventing a general immune response to sperm. Intra-epithelial lymphocytes in the surface and SST epithelia may function in a similar manner. Hypothetically, a breakdown in this system would lead to an immune response directed against oviductal sperm resulting in a lower number of sperm reaching the SST and consequently lower hen fertility.

H.P. Van Krey: Evidence of degenerating spermatozoa within uterovaginal sperm-storage tubules is limited. Could you explain the apparent lack of degenerative spermatozoal debri within a tubule despite prolonged storage?

Author: The mechanism by which viable sperm are released from the SST to ascend the oviduct to the site of fertilization and the mechanism by which dead or senescent sperm are released from the SST (hence the absence of degenerating sperm) to be expelled from the vagina (Bakst, 1981), are probably the same. However, the precise mechanism(s) of sperm release from the SST is not known. Hypothetically, once resident sperm either lose their ability to remain agglutinated or randomly disengage from the sperm mass, the liberated sperm are swept out of the SST (see Van Krey et al., 1981), possibly through negative pressure created by ciliary activity at the opening of the SST. The SST lumen has been observed with accumulation of periodic-acid Schiff-positive material which is assumed to have originated from the surface secretory epithelial cells.

H.P. Van Krey: Could you share with us your thoughts regarding the cause(s) of the relatively poor fertility seen in turkey breeder hens late in an egg production year?

Author: Disregarding such possible factors as declining semen quality, faults in the artificial insemination technique and general management problems, the decline in hen fertility late in the egg production season is of unknown etiology. Hens are inseminated 10 to 15 times during a 22 wk egg production season. I speculate that sperm may gain access to lymphoid tissue in the oviduct either through damage to the oviductal mucosa during insemination or through some yet undefined mechanism. Furthermore, sperm may reach the cecal tonsil or proctodeal gland, known sites of lymphoid cell accumulations. Repeated inseminations (innoculations?) may result in a local immune response which would possibly prevent sperm from reaching the SST and result in lowered fertility.

B. Howarth, Jr.: I agree with Lake's (1975) observation that prolonged sperm-storage in birds obviates the need for the synchronization of copulation and ovulation by the female during the so-called "fertile-period". Considering, however, "the anatomical basis for sperm-storage is the sperm-storage tubules located at the utero-vaginal junction", what synchronizes the presence of sperm in the infundibulum (site of fertilization) with ovulation? The short fertilizable life span of the hen's ovum would necessitate the presence of viable spermatozoa in the infundibulum at the time of ovulation. Would the author care to speculate on the functional significance of the "secondary sperm-storage sites" in the infundibulum of the chicken and turkey oviduct?

Author: If as some investigators suggest, sperm are released throughout the 26 h ovulatory cycle (the

time interval between two successive ovulations) then viable sperm will ascend the oviduct and reside within the secondary sperm-storage sites at the distal infundibulum. Sperm released from the SST must keep the distal infundibulum, the site of fertilization, populated with sperm in order to insure successful fertilization. Other investigators suggest that sperm release from the SST is associated with ovulation and / or oviposition. If this is true, then sperm released from the SST following oviposition must ascend the oviduct within the 30-40 min interval between oviposition and the next ovulation. It is assumed that if an ovulation does not take place sperm reside in the distal infundibulum until the next ovum is ovulated.

B. Howarth, Jr.: How are sperm released from the sperm-storage tubules located at the utero-vaginal junction in the hen?

Author: Van Krey et al. (1981) presented a tenable model of sperm release from the SST (see paper). Squash preparations of SST-containing mucosa reveal vigorously beating cilia around the opening of the SST. Possibly this ciliary activity is sufficient to augment the movement of fluid and disengage sperm up and out of the SST lumen.