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Turkey Sperm Reside in the Tubular Glands in the Urodeum Following Artificial Insemination[1]

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ABSTRACT A turkey hen in egg production requires 48 h after the last insemination to maximize the number of sperm in the uterovaginal junction sperm-storage tubules. Where the sperm that continue to fill the oviductal sperm-storage sites during this 48-h period reside remains unknown. Histological sections of the juncture of the vagina with the urodeum, the central compartment of the cloaca, revealed deep tubular glands containing periodic acid-Schiff-positive secretory material. When examined 36 h after the last artificial insemination, sperm were observed in the lumen of the tubular glands associated with the urodeum. We suggest that sperm reside in the tubular glands within the urodeum and are released in association with the secretory activity of the tubular glands. These sperm then may ascend the vagina to continue to populate the sperm-storage tubules. Alternatively, the sperm in the tubular glands of the urodeum may be evidence of spermatorrhea and have no functional role in the fertilization process.

Key words: avian, poultry, turkey, oviduct, cloaca

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INTRODUCTION

In the mature turkey hen, the number of sperm residing within the sperm-storage tubules (**SST**) is quite small relative to the number of sperm artificially inseminated, which ranges from 200 to 350×10^6 sperm (Brillard and Bakst, 1990). In hens inseminated before the onset of egg production, only 4 h is necessary for the maximum number of sperm, about 4.1×10^6, to populate the SST (Brillard and Bakst, 1990). In contrast, if the hen was inseminated after the onset of egg production, it required at least 48 h for the maximum number of sperm, about 2×10^6, to enter the SST. The question not addressed by Brillard and Bakst (1990) was where in the vagina, or elsewhere, do the sperm that continue to populate the SST over the 48-h period reside?

The terminal segment of the avian oviduct is the vagina. In Galliformes, not only is the vagina the conduit between the uterus and the cloaca for the hard-shelled egg, but the vagina also deposits the cuticle around the shell at the time of oviposition. The vagina is also the site of semen transfer in artificial insemination (**AI**). Whether semen is deposited directly in the vagina during natural mating or the cloaca remains equivocal (Lake, 1981). This is because Galliform males do not possess a true intromittent phallus.

There is little information regarding the histological relationships between the cloacal compartments and the junctures of the digestive and urogenital tracts. Oliveira et al. (2004) did a comparative study examining the location of the ureteral openings in the cloaca in 67 avian species and found some variation in the precise location of the ureteral opening. However, in all species they examined, the vagina terminated at the urodeum. Dahm et al. (1980) focused exclusively on the chicken and observed the ciliated epithelium of the vagina merge with the secretory epithelium of the urodeum on 1 border and the secretory epithelium of the proctodeum on the other border.

The possibility that the cloaca may serve as a reserve for sperm has yet to be investigated. In the following study, we examined the histology of the juncture of the vagina and urodeum in turkey hens within 36 h of AI.

MATERIALS AND METHODS

Large White turkey poults were raised according to the guidelines suggested by Nicholas Turkeys (Nicholas Turkeys, Lewisburg, WV). Toms and hens were photostimulated by increasing the duration of light from 6 h (18 h dark) to 14 h (10 h dark) at 26 and 28 wk, respectively.

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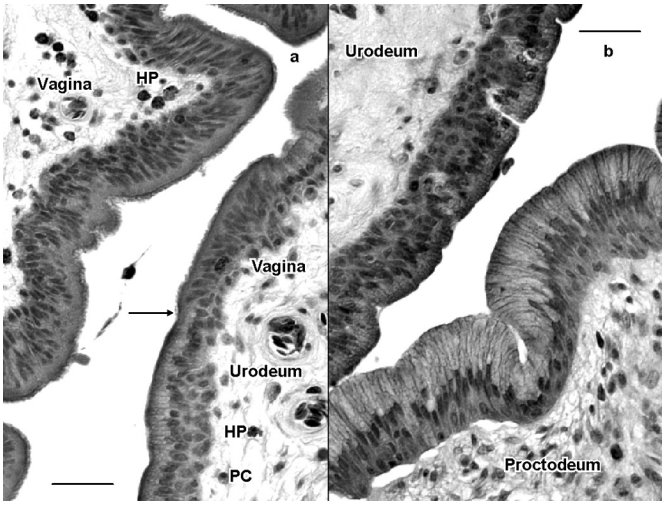

Figure 1. a) The juncture of the vagina with the urodeum (arrow) is abrupt. The ciliated epithelium of the vagina contrasts sharply with the nonciliated epithelium of the urodeum. The lamina propria underlying each epithelium is characterized by heterophils (HP), plasma cells (PC), and connective tissue elements. Bar = 25 μm. b) The ventral aspect of the proctodeum is lined by a columnar secretory epithelium with no conspicuous basal cell layer. The urodeum is also lined by a secretory epithelium, but it has a distinct basal cell layer. Bar = 25 μm.

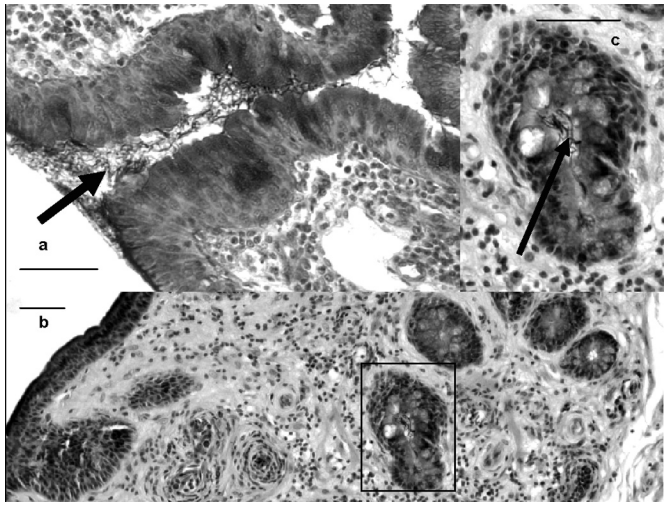

Figure 2. a) This deep invagination of the surface epithelium of the urodeum, which is partially covered by an aggregate of sperm, leads to the tubular glands. Densely distributed sperm are barely discernible both at the orifice of the tubular gland (arrow) and deep within the invagination. Scattered in the lamina propria are numerous lymphocytes. Secretory material in the cells lining the invagination is strongly periodic acid-Schiff-positive. Bar = 25 μm. b) A section revealing several cross-sections of tubular glands lined by a secretory epithelium may contain resident sperm. The framed cross-section of a tubular gland contains sperm and is observed at higher magnification in panel c. Bar = 25 μm. c) At increased magnification, the cross-section of the tubular gland observed in panel b clearly houses sperm in its lumen (arrow). Bar = 25 μm.

By 3 to 4 wk after the onset of photostimulation, semen was collected manually, diluted with an equal part (vol/vol) of Beltsville Poultry Semen Extender (Continental Plastic, Delavan, WI), and inseminated into the hens within 1 h of collection. Only visually clean, viscous, pearly white semen was used for AI. The 2 initial AI were performed 48 h apart before the onset of egg production (30 to 31 wk of age) with 300×10^6 sperm. Each week thereafter, hens were inseminated once with the same number of sperm.

Turkey hens used in this study (n = 6), which were between 34 and 40 wk of age, were euthanized by cervical dislocation 36 h after the last AI. Care was taken to isolate the cloaca, uterus, and vagina as 1 segment. The vagina was stripped of its connective tissue encasement to permit access to the uterovaginal junction (**UVJ**). The UVJ containing SST, confirmed by stereomicroscopy, and samples from the anterior, mid, and distal regions of the vagina were fixed in neutral-buffered formalin. Cloacal tissue around the juncture of the vagina with the urodeum and ventral proctodeum, as well as tissue from the infundibulum-magnum junction, were also collected and fixed. After 48 to 72 h in fixative, the tissues were processed for histology. Paraffin sections were cut at 5 to 7 μm and stained with hematoxylin and eosin or by periodic acid-Schiff (**PAS**; Sigma, St. Louis, MO; Luna, 1968).

RESULTS

In all hens examined within 36 h of the last AI, sperm were observed in the SST and, more rarely, in tubular glands in the transition region at the infundibulum-magnum junction. Luminal sperm were rarely found in the vagina or UVJ.

Depending on the orientation of the tissue in the section, the juncture of the vagina with the cloaca was lined by 1 of 3 surface epithelia. The vagina was lined with a pseudostratified columnar ciliated epithelium that had a light to moderate PAS-positive staining in the apical cytoplasm (Figure 1a). Like the urodeum, the surface epithelium of the vagina had a distinct layer of basal cells that may or may not have extended to the vaginal lumen (Figure 1a,b).

The vagina and the urodeum merged abruptly with no transitional cells (Figure 1a). Lining the urodeum was a pseudostratified, columnar, nonciliated, secretory epithelium (Figures 1a,b, Figure 2a). In some areas, the lining epithelium of the urodeum appeared to consist of a basal layer of cuboidal cells with an upper layer of columnar secretory cells. The apical cytoplasm of the columnar secretory cells was lightly PAS positive. Random deep invaginations of the surface of the urodeum gave rise to branching tubular glands (Figure 2a,b). The secretory epithelium lining the tubular glands was strongly PAS positive. Sperm were often scattered throughout the surface invaginations of the urodeum and observed in the lumen of the deep tubular glands (Figure 2a,b,c). These sperm did not appear to have any unique orientation. Sperm observed in the lumen of the urodeum were often in clusters, possibly bound by secretory material.

The ventral aspect of the proctodeum that merged with the vagina consisted of a columnar secretory epithelium and lacked a clearly discernible layer of basal cells (Figure 1b). The secretory material occupied the apical half of the

columnar cells and was moderately PAS positive. Tubular glands in the lamina propria originating as surface invaginations of the surface epithelium of the proctodeum were sparse and when observed, rarely possessed sperm.

Other than the presence of the tubular glands both in the urodeum, and to a lesser extent in the proctodeum, the cellular composition of the lamina propria associated with each of the 3 mucosae was quite similar. In addition to the connective tissue cells and elements, there were variable concentrations of lymphocytes. Large aggregates of lymphocytes were particularly evident in the urodeum (Figure 2a,b) and to a lesser extent in the proctodeum and vagina. Heterophils, plasma cells, and macrophages were also present in the lamina propria underlying the 3 epithelia.

DISCUSSION

The histology of the juncture of the turkey vagina with the cloaca is similar to that described by Dahm et al. (1980) for the chicken with a few important exceptions. Dahm et al. (1980) failed to note the presence of tubular glands in the urodeum. Interestingly, these tubular glands are clearly apparent in Figure 10 of Dahm et al. (1980). Dahm et al. (1980) described crypts as possibly containing uric acid, but they failed to describe the tubular glands extending into the lamina propria. In addition, what were described as motile cells by Dahm et al. (1980) appear to be lymphocytes. Bakst and Cecil (1983) examined the histology of turkey papillae, the wart-like termini of the ductus deferens in the urodeum, and noted the surface epithelium was a pseudostratified columnar epithelium atop a layer of basal cells. The observations in this study confirmed that the surface lining the turkey female urodeum is nearly identical to that seen lining the papillae.

Of particular interest to us was the observation that the tubular glands in the urodeum of the turkey housed sperm up to 36 h after the last insemination. As noted in the introduction, turkey hens inseminated after the onset of egg production required up to 48 h to maximize their numbers in the SST (Brillard and Bakst, 1990). Before the observations in this study, we could not find where the sperm resided in that 48-h period between insemination and maximum filling of the SST. We propose here that the tubular glands in the urodeum, and possibly elsewhere in the cloaca, serve as a temporary storage site for sperm. We state temporary storage, because distension of the cloaca due to oviposition or fecal expulsion may initiate the release of secretory material from the tubular glands and, in turn, the release of some resident sperm. Once in the urodeum, sperm may ascend the vagina and continue to populate the SST, thus accounting for the persistent filling of the SST over a 48-h interval from AI.

With Galliformes that copulate naturally, the role of the urodeal tubular glands with respect to sperm storage may be even more significant than with hens that were subject to AI. With AI, semen is deposited 2 to 4 cm into the vagina. However, given the anatomy of the phallus nonprotrudens and absence of true intromission at copulation, sperm may be transferred into the cloaca and not directly into the vagina. Although it is doubtful that a sperm-selection process is associated with the cloaca, sperm entering the urodeal tubular glands could be temporarily sheltered, affording a more gradual transfer of sperm into the vagina over time. Lower sperm numbers entering the vagina over a longer period of time may increase the efficiency of both sperm selection in the vagina and sperm transport to the UVJ-SST.

An alternative explanation for the presence of sperm in the urodeal tubular glands is spermatorrhea, the loss of semen from the vagina, here after AI. Howarth (1971) found that within 15 min of AI, 57.6% of the sperm inseminated was excreted into the cloaca. Some of the sperm in the urodeum may also represent a subpopulation of nonviable sperm released from the SST and transported back through the vagina to be expelled into the cloaca (Bakst, 1981).

To summarize, tubular glands were found in the lamina propria of the turkey urodeum, and, to a lesser extent, the lamina propria of the ventral proctodeum. We proposed that sperm in the lumen of these tubular glands were carried out with the secretory material secreted from the tubular glands and possibly ascended the vagina to augment the population of sperm residing in the SST.

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