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PHYSIOLOGY, ENDOCRINOLOGY, AND REPRODUCTION

Morphology of the turkey vagina with and without an egg mass in the uterus¹

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ABSTRACT In this study we examined the gross anatomy of the uterus and vagina in turkeys in egg production. With no uterine egg mass, removal of the tunica serosa that enclosed the uterus revealed deep periodic in-folding of the muscularis transversely circumscribing the sac-like segment. When the connective tissue embracing the neutral buffered formalin fixed vagina was completely teased free, the exposed tubular segment was shaped as a counter-clockwise spiral or as a series of angular, random bends. The uterovaginal junction was flush with the uterine mucosa or projected slightly into the uterine lumen. With a uterine egg mass, the deep in-foldings of the uterus were abolished. The only alteration to the morphology of the vagina was that the

uterovaginal junction appeared dilated and pressed into its juncture with the uterus. Whether an egg mass was present or not, uterovaginal junction folds that projected into the uterus possessed sperm storage tubules. An egg mass in the uterus compressed the uterovaginal junction folds, and its mucosa became contiguous with the uterine mucosa. Finally, from an evolutionary perspective, in the turkey and possibly other species possessing a nonintromittent phallus, vaginal pleomorphism may have been driven primarily by the need to accommodate the overall length of the vagina in a limited abdominal space and to a lesser extent on sexual selection.

Key words: ave, genitalia, oviduct, anatomy

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INTRODUCTION

In birds, the vagina is the terminal segment of the oviduct, and anatomically as well as functionally it remains poorly understood. In poultry, this may be a consequence of the vagina contributing little to egg formation or because of the difficulty in isolating the vagina; particularly its most cranial region, the uterovaginal junction (**UVJ**). However, since the early 1990s, interest in the avian vagina has increased because its critical role in sperm selection, sperm storage, and ultimately sustained fertility and paternity (Bakst et al., 1994; Wishart and Horrocks, 2000; Stepinska and Bakst, 2007).

In chickens and turkey, the vagina has been described as an S-shaped segment (Aitken, 1971) serving as the conduit between the uterus and cloaca at the time of oviposition (Bakst et al., 1994; Jacobs and Bakst, 2007). Recent work by Brennan et al. (2007) described the unique anatomical characteristics of waterfowl genitalia and the implications of these morphological character-

istics on sexual selection. Specifically, Brennan et al. (2007) were the first to describe the complex anatomy of the waterfowl vagina. Brennan et al. (2007) also correlated the length and configuration of the vagina to the morphology of the male's intromittent organ and subsequently arrived at explanations describing the co-evolution of the waterfowl genitalia and the female's cryptic response (see Eberhard, 1996) to extra-pair copulations.

In the following study, we will describe the gross anatomy of the turkey vagina. In doing so we will show that the turkey vagina has a complex anatomical configuration that is unlikely related from an evolutionary perspective to the male turkey's phallus nonprotrudens (nonintromittent organ; for description, see Bahr and Bakst, 1987). We suggest that the configuration of the turkey vagina was more an evolutionary adaptation to its functions and the volume constraints afforded by the abdominal cavity.

MATERIALS AND METHODS

Mature Large White turkey hens (Hybrid Turkeys, Kitchener, Canada) in egg production (36 to 44 wk old) and with (n = 10) or without (n = 12) a hard-shelled egg mass (the egg mass is the ovum and accompanying investments before oviposition) in the uterus were

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killed by cervical dislocation. The oviduct was carefully removed, and the distal isthmus, uterus, vagina, cloaca, and coprodeum were immersed as one piece in 10% neutral buffered formalin (NBF). After 1 wk in the NBF fixative, the oviducts were transferred in toto to 70% ethanol, washed 3 times with 70% ethanol over 48 h, and then stored in 70% ethanol until dissection. Photographs were taken with a digital camera (Mavica, Sony MVC-FD91, San Jose, CA) at each stage of the dissection.

Procedures for the removal of the connective tissue enveloping the UVJ are described elsewhere (Bakst, 1987). To visualize the luminal mucosa, sections of the uterus and vagina were excised from the NBF-fixed specimens without altering the overall morphology of the segments. To determine the presence or absence of sperm-storage tubules (SST) in the high narrow longitudinally oriented mucosal folds of the UVJ, small pieces of the folds were removed, placed on microscope slide, teased apart, and examined using phase contrast or interference contrast microscopy. After removal of its enveloping connective tissue, the length of the vagina, defined here as the vagina-urodeum transition to the vagina-uterus transition, was estimated by the method of Brennan et al. (2007). If present, the orientation of

the spirals (clockwise or counter clockwise) was determined to be in the direction of the cloaca to the UVJ.

RESULTS

Macroscopically, the sac-like uterus was bound by a tough, opaque tunica serosa that merged with the connective tissue masking the convolutions and angularity of the vagina (Figure 1, panel 1). Attached to the ventral aspect of the uterus was the ventral ligament that toward the vagina gave rise to the muscular cord. This cord inserted into the connective tissue enveloping the proximal end of the vagina (Figure 1, panel 1). The coprodeum, the most cranial compartment of the cloaca, was also observed merging with the connective tissue that masked the juncture of the vagina and urodeum, the central compartment of the cloaca. The close apposition of the vagina and coprodeum was evident as each continued separately into the urodeum (Figure 1, panel 2). We found that in isolating fresh vaginal tissue, it is important to avoid cutting the coprodeum and prudent to remove or divert its contents.

In the absence of an egg mass and after stripping away the tunica serosa, 2- to 3-mm-wide folds prominently circumscribed the uterus (Figure 1, panels 2 and

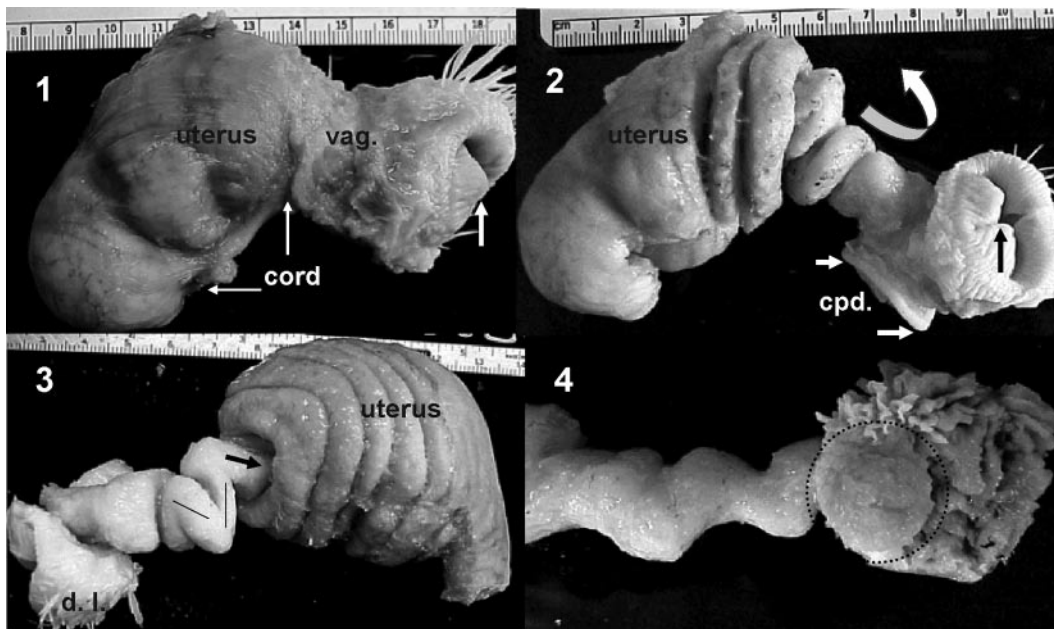


Figure 1. Panel 1). Observed with no egg mass is the uterus, vagina (vag.) bound in connective tissue, and cloacal lips with the ventral lip highlighted (arrow). The muscular cord originated from the ventral ligament near the cranial end of the uterus and embeds in the connective tissue at the cranial end of the vagina. The thick, nearly opaque, tunica serosa surrounds the uterus and is contiguous with the connective tissue enveloping the vagina. Panel 2). The same specimen as panel 1 except it has been rotated slightly on its long axis to reveal the coprodeum (cpd.), the most proximal segment of the cloaca. The coprodeum is closely associated with the vagina and both open into the urodeum. The cloaca is observed with the black arrow highlighting its ventral lip. The tunica surrounding the distal 2 circumferential folds of the uterus as well as the connective tissue enveloping the vagina has been removed, exposing the pleomorphic character of these segments. Two counter-clockwise (curved arrow) spirals of the vagina are observed as a part of a third fold appears to enter the uterus. Panel 3). Another specimen without an egg mass that had been stripped of the tunica serosa and connective tissue. Unlike panel 2, the configuration of the vagina consisted of angular bends (straight lines). The arrow shows the insertion of the uterovaginal junction (UVJ) into the uterine lumen. Five circumferential folds of the uterus are clearly observed, and a sixth is partially hidden adjacent to the tubular portion of the uterus. d. l. = dorsal lip. Panel 4). Here the spiral curves are flatter than seen in panel 2. Most of the uterus was removed to expose the slight extension (within the dotted circle) of the UVJ into the uterine lumen. These UVJ folds contain sperm-storage tubules.

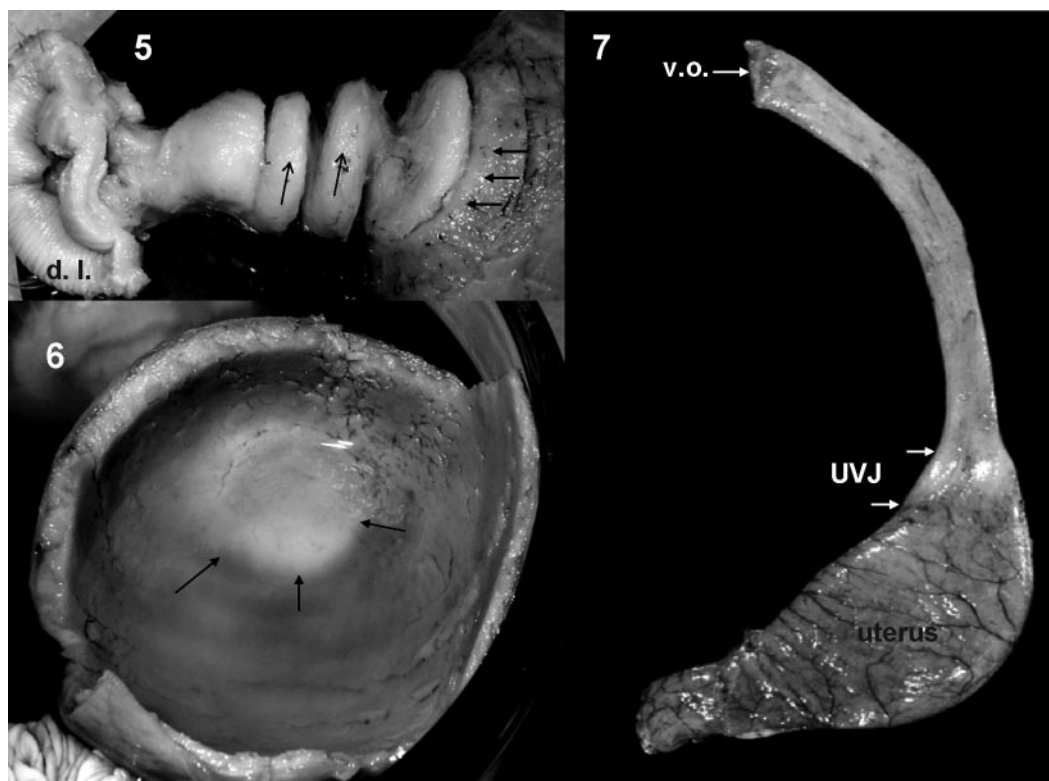


Figure 2. Panels 5 and 6). Two views of the same specimen that had a hard-shelled egg mass removed from the uterus. Panel 5 clearly shows 2 of the counter clockwise (vertical arrows) spirals of the vagina. A less pronounced spiral associated with the uterovaginal junction (UVJ) is barely separated from the uterus. The 3 horizontal arrows point to the border marking the paler uterovaginal junction and the darker uterus. This line is more conspicuous in panel 6 where part of the uterus has been removed, exposing its lumen and a clear view of the paler folds of the UVJ (arrows). The cloaca's more pronounced dorsal lip (d. l.) is observed adjacent to the ventral lip (panel 5). Panel 7. An unfixed, fresh specimen shows the uterus enveloped in its tunica serosa and the vagina and UVJ stripped of their connective tissue investments. The plasticity of fresh, unfixed vagina and UVJ was apparent because no morphological elaborations were observed. v.o. = vaginal orifice.

3). The most distal uterine fold encompassed the UVJ; the UVJ appeared to project slightly into the uterine lumen (Figure 1, panel 3). When the uterine and vaginal lumina were exposed, the UVJ was observed flush with the uterine mucosa or projecting 1 to 3 mm into the uterine lumen (Figure 1, panel 4). The high narrow folds of the UVJ that projected into the uterine lumen possessed SST.

After removal of the tunica the external surface of uteri fixed with an egg mass lacked the deep pronounced circumferential folds and had a smooth appearance (Figure 2, panel 5). Removal of the connective tissue enveloping the UVJ revealed a broad, whitish, flattened fold against the uterine wall (Figure 2, panel 5). After removing the egg mass and exposing the uterine lumen, the UVJ mucosa appeared contiguous with the distended luminal uterine mucosa (Figure 2, panel 6). Here the UVJ mucosal folds formed a whitish disc about 20 to 25 mm in diameter that clearly contrasted with the contiguous darker, cordovan-colored, uterine mucosal folds. These UVJ folds possessed SST.

Removal of the connective tissue enveloping the formaldehyde fixed vaginas revealed a tubular segment of varying lengths (40 to 100 mm) exhibiting spiral-like curves (Figure 1, panels 2 and 4; Figure 2, panel 5) or angular bends (Figure 1, panel 3) along its length. One

to 4 counter-clockwise spirals were predominantly localized in the central area of the vagina, whereas angular bends of the vaginal segment appeared not to have a pattern. In contrast, the spiraling curves of the fresh, unfixed vagina become flaccid and then abolished as the enveloping connective tissue is removed. Whether fixed or unfixed, neither straight, simple tube-like vaginas nor blind-sacs branching from the vagina were observed. When cut transversely the vaginal mucosal folds were tightly apposed to each other except at the dilation of the UVJ and the vaginal orifice at the urodeum. The vaginal mucosa projected a short distance into the urodeum where it merged with the mucosal folds of the urodeum.

DISCUSSION

The anatomy of the uterus and vagina following fixation and removal of the surrounding tunica serosa of the uterus and connective tissue of the vagina reveals a pleomorphism similar to that recently described for the waterfowl vagina (Brennan et al., 2007). However, 2 features differentiated the vagina of the turkey from that of the waterfowl: no blind sacs in the turkey oviduct, and the counter-clockwise spirals of the turkey vagina. The variation we observed in the length of the

vagina in this study when compared with previous reports that used unfixed tissues (Bakst, 1987; Brillard and Bakst, 1990) was most likely due to the shrinkage and hardening effects of NBF fixation on tissues in contrast to the flaccid and pliable tone of unfixed vaginal tissue. The plasticity of the unfixed vagina is evident in Figure 2 (panel 7) because there is no indication of any morphological elaborations associated with the manually straightened turkey vagina.

There are several known functions of the turkey vagina that are applicable to other avian species in egg production. The vagina serves as a conduit for the egg mass at the time of oviposition. Upon passage of the egg mass through the vagina, the luminal surface epithelial secretory cells secrete a mucin-like substance that fills the shell pores and coats the egg shell. The cuticle functions as a barrier to egg water loss and the penetration of microorganisms through the shell pores (Solomon et al., 1994).

Just as the cuticle inhibits contamination of the laid egg, the vagina appears to function as a physical and physiological barrier to contamination of the oviduct. This is a necessity given the continuity of the vagina with the urodeum, a compartment that also receives the coprodeum and subsequently is exposed to fecal debris and microorganisms. The vaginal length, the high, tightly apposed luminal mucosal folds, and the observed spirals and angular bends would contribute to obstructing particulates and possible aqueous contamination from ascending the vagina. One would also think that the length of the vagina should be as long as or longer than the length of the egg mass being oviposited. If shorter, the distended vagina at oviposition would permit free access for debris and foreign organisms to the uterus.

Histologically, the vaginal epithelium and subepithelial luminal mucosa are heavily populated with lymphocytes, macrophages, and plasma cells, suggesting a high degree of immunological competence (Bakst, 1987; Wishart and Horrocks, 2000; Froman, 2003). Histologically, the distribution of these cell types in the vaginal mucosa is reminiscent of the gut-associated lymphoid tissue described in birds (Befus et al., 1980). Sperm selection by the vagina also appears to have an immunological component (reviewed by Wishart and Horrocks, 2000). Here again, increasing length of the vagina affords more surface area to initiate and maintain an immunological response to targeted microorganisms or sperm that may be recognized as immunologically unfit.

Considerable work has been published since the early 1990s on the role of the vagina in sperm selection and storage at the UVJ, the site of the SST. This will not be covered here because it has been reviewed by others (Bakst et al., 1994; Wishart and Horrocks, 2000; Froman, 2003; Stepinska and Bakst, 2007). Most recently, Bakst and Akuffo (2008) have suggested a local control mechanism behind cryptic sperm selection in the turkey vagina based on the presence of serotonin-positive

surface epithelial cells. In other systems and species, neuropeptides have been shown to increase cilia beat activity and sperm motility, respectively (Atherton et al., 1980).

Before this study, it was understood that the SST were located in the UVJ at the cranial end of the vagina. Of significance is our observation that the morphology of the UVJ folds vary with the stage of the ovulatory cycle. With an empty oviduct (free of an egg mass), the UVJ mucosa folds are clearly discernible projecting slightly into the uterus. However, with an egg mass in the uterus, the UVJ mucosa becomes compressed and clearly contiguous with the uterine mucosa.

The presence or absence of a uterine egg mass influencing the morphology of the UVJ folds containing the SST has implications on sperm acceptance into the SST and the fate of sperm upon egress from the SST. Brillard and Bakst (1990) showed that the SST of hens inseminated within 1 wk before the expected onset of egg production were filled faster (4 h vs. 2 d) and possessed more sperm (4.1 million vs. 2.0 million) than the SST of hens inseminated after the onset of egg production. The morphology of UVJ folds would influence the number of sperm entering the SST. With an empty oviduct, more sperm would rapidly fill the unobstructed SST localized to the UVJ folds than with that the compressed folds containing an uterine egg mass. With an egg mass, the distention of the UVJ folds within the uterine luminal mucosa coupled with the movement of the uterine musculature, and uterine fluids dispersing the selected sperm make optimal SST filling an impossibility and also explains why filling of the SST requires 48 h after insemination.

Egress of sperm from the SST would likewise be influenced by the presence or absence of an egg mass in the uterus. Bakst et al. (1994) speculated that substances in the SST lumen may function to stabilize or possibly to decapacitate resident sperm, and the uterine fluid, presumably rich in calcium, would act to activate or possibly to capacitate the released sperm.

From the discussion above it is evident that segment length is critical for proper immunological function and necessary to accommodate the extensive sperm selection process. One may explain the coiled and folded appearance of the vagina as an evolutionary mechanism to condense this vital segment into limited abdominal cavity space. Furthermore, one can also assume that the dense connective tissue enveloping the vagina prevents a prolapse or a passive, gravity-induced movement of the egg mass into the vagina before the oviposition because there is no muscular sphincter at the UVJ. Interestingly, the coiled configuration of the vagina was reminiscent of variation in individual SST morphology (Bakst, 1992).

As cited by Brennan et al. (2007), only 3% of known avian males possess a phallus that can be considered an intromittent organ (Briskie and Montgomerie, 1997). Given that in the turkey, as in other galliformes, the phallus is a nonintromittent organ, one is drawn to the

conclusion that functionality rather than morphological compatibility between the sexes is the evolutionary basis of the vagina's pleomorphism. Although Brennan et al. (2007) suggested that incompatibility between the same species male and female genitalia minimizes the impact of extra-pair copulations; this same physical incompatibility accompanies breeding pairs that successfully mate. In the latter situation, receptivity of the female most likely involves a relaxation of the vaginal musculature and associated structures to facilitate male intromission. In contrast, in episodes of extra-pair copulation, the vaginal musculature as well as the ventral ligament and muscular cord may constrict and impede intromission by the violating male.

To conclude, the turkey vagina had previously been described as an S-shaped or coiled segment, but these descriptions were not documented in the literature. We described the turkey vagina's morphological complexity and suggest that its length and pleomorphism is similar to that described for some waterfowl (Brennan et al., 2007). However, unlike the vagina observed in waterfowl, from an evolutionary perspective, we suggest that the anatomical characteristics of vaginas from a commercial line of turkeys were primarily driven by the need to accommodate the length of the vagina in limited abdominal space. It would be interesting to determine if vaginas from wild turkeys, not subject to intense genetic selection for increased BW, have similar anatomical configurations.

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