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Gross and Histomorphological Study of the Ovary and Oviduct of Turkey Hen with Especial Emphasis on the Sperm-Host Gland

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A B S T R A C T

Turkey bird is one of the popular poultry species which is reared primarily for meat production and considered as one of the major sources of animal protein. With such importance of this species, this study was designed to investigate the gross and histomorphology of the ovary and oviduct with especial emphasis on sperm-host glands of the turkey hen involving ten mature female turkeys (*Meleagris gallopavo)*. The present study highlighted the distribution pattern of sperm-host glands (SHGs) in the oviduct of turkey hen that has a potential role in producing a fertile egg in poultry industries. The oviduct of turkey consists of the infundibulum, magnum, isthmus, uterus, and vagina which are sole distributors for making nutrition enriched egg. The tissue samples were collected from the ovary, different segments of the oviduct and especially uterovaginal junction (UVJ) and infundiomagnal junction of the oviduct. The ovaries and the oviducts were dissected and fixed in Bouins solution and processed for a light microscopic study. Histologically, the left ovary of turkey consisted of an outer cortex and inner medulla, with different stages of follicles. In all areas of the oviduct except the infundibulum and vagina, the tunica mucosa epithelium was lined with ciliated pseudo stratified columnar epithelium, and the lamina propria-submucosa contained branched tubular glands. Sperm-storage tubules were observed in the uterovaginal junction and infundibulo-magnum junction. These tubules were mostly branched, slightly coiled and extended into the lamina propria from the bases of the mucosal folds. These glands had proximal and distal parts; the proximal part was lined by pseudostratified columnar epithelium and distal part by non-ciliated simple columnar epithelium. The number of sperm host glands was more at uterovaginal junction than infundibulomagnal junction. The sperm-host glands might play a functional role in the storage and release of spermatozoa from the SHGs in response to oviposition or ovulation. The results would help poultry scientists and farmers in developing effective disease control and growth strategies.

Keywords: turkey, ovary, oviduct, sperm host gland, histomorphology

INTRODUCTION

The domestic turkey (*Meleagris gallopavo*) is a large poultry species in the *Meleagris* genus that is related to the Wild Turkey (1). Commercial turkey farming is a profitable business idea in Bangladesh. Turkey grows faster as broiler chickens and became suitable for slaughter purposes within a very short time. Turkey farming for meat and egg production is very popular in Bangladesh (2). However, due to the seriousness of avian influenza, the poultry industry (especially commercial broiler and layer chickens) is in the line of extinction. Thus, it is a crying need

to search for the alternative protein source to meet the increasing demand (3). Turkey birds have the potential to be a competitive meat production alternative to livestock (4). Turkey meat may be one of the best options for alternative protein source in Bangladesh. Turkey production is a major and profitable agricultural sector, with growing global demand for its products (5), and they can also adapt to a wide variety of climatic conditions (6). (7) Stated that consumption of turkeys and broilers as white meat rose worldwide, and a similar trend existed in developing countries. If the broiler meat industry is experiencing increased disease and declining taste, turkey meat may be a suitable alternative for customers. Therefore, it could be an effective alternative source of protein. However, turkey production has not been fully exploited in Bangladesh despite its vast potential over other poultry species.

However, there is no organized market for turkey in Bangladesh. In Bangladesh, the weight of available adult tom, hen, and egg was lower than in developing countries. This may be attributed to the lighter varieties of turkeys raised by Bangladeshi farmers. This might be because of lighter varieties of turkeys reared by the farmers of Bangladesh. The hatchability of eggs was low because of lower fertility, including insufficient knowledge of farmers on turkey breeding and egg incubating procedure. Although commercial livestock species are completely dependent upon artificial insemination (AI) for fertile egg production, none of them are the respondent farmers. The problem of unfertilized eggs is long identified as one of the most critical factors limiting the success of breeding programs, which are ranged from 10.0 to 98.2% (8).

The primary site of sperm storage in the bird oviduct is the sperm-host glands. These glands can store spermatozoa for few months after a single mating; subsequently from here, the spermatozoa are released gradually and fertilize the ovum. Sperm-host glands are the tubular structure and distributed in the lamina propria of the infundibulum, uterovaginal junction, and the vagina (9-11). It plays an essential role in the reproduction of turkey birds. However, in a reproductive perspective, no research is carried out regarding the distribution pattern of sperm-host glands, the gross-morphometry, histochemistry of oviduct of Turkey in Bangladesh. Therefore, the present research carried out to understand the gross and histological architecture of the ovary and oviduct with special emphasis on the sperm-host glands of turkey hen for reproductive perspective in Bangladesh that may provide valuable information to the poultry scientist, anatomist, and pathologist.

MATERIALS AND METHODS

Experimental Location and Animal Ethics

The current research was carried out in the Department of Anatomy and Histology of Bangladesh Agricultural University, Mymensingh. The necessary permissions were obtained from the Bangladesh Agricultural University Animal Ethics Committee.

Experimental Birds

Adult female turkeys (*Meleagris gallopavo*) of three lines (n=10) were used. These turkeys were purchased from the village farmers and nearby farms close to Bangladesh Agricultural University, Mymensingh. The age of the birds ranged from 8 to 10 months which were based on the farmers' information. The birds were reared for 1 week in a nearby shed supplying layer feed and added libitum water before they were sacrificed.

Sample Collection

Turkeys were sacrificed in this study by cervical subluxation. Every bird was dissected according to normal anatomical procedures. The whole ovary and oviduct was dissected and stretched on a tray. In the trial, oviducts that were clean of gross pathological disease were used. The oviducts were used in the experiment.

Gross Study

In the gross observation, the gross weight, length, and width of different parts of the left oviduct of turkey were taken into deliberation. The color of the samples was recorded by visual inspection. The weight was measured in grams by electronic balance. The length and width of the different parts of the oviduct were measured by Mitutoyo Digital Slide Calipers in scale of cm.

Tissues Preparation for Histomorphological Study

After collecting the ovary and oviducts, segments of the oviducts were separated. For the sperm host gland, the tissue samples were collected from the uterovaginal junction (UVJ) and infundiomagnal junction of the oviduct. The ovaries and oviducts were dissected. Small pieces of each segment were fixed in Bouins fluid for 24 hours. Following fixation, the samples were dehydrated for 2-3 hours in a sequence of ascending alcohol grades (70 percent, 80 percent, 90 percent, 95 percent, 100 percent I, 100 percent II, and 100 percent III). The tissues were then infiltrated with paraffin after cleared in three changes of xylene, each for 30 minutes. The tissues were embedded with paraffin bath at 58-60 °C for (time), and the embedded paraffin blocks were then added to the block holder. A sliding microtome was used to section tissues at a thickness of 6 µm (MIC 509, Euromex, Japan). After sectioning, the sections were floated on a lukewarm water floatation bath (KF-WS-100 Tissue Flotation Water Bath) for stretching, and then the paraffin sections were mounted on slides glass using an adhesive (egg albumins) and dried 6 to 24 hours on a hot plate of slide warmer boxes. Tissue sections were

stained with Mayers Hematoxylin and Eosin method to study the histological structures of the ovary and oviduct. Finally, the slides were mounted with DPX, which was kept on top of the tissue section for 24 hours to dry. After all these preparations of slides, photographs were taken with an Olympus CX23 microscope equipped with a camera. Under the microscope, the histological layers of the segments of ovary and the oviduct, such as tunica mucosa, submucosa and muscularis were examined. The size and shape of the epithelium and the height of the mucosal folds of the different segments of the oviduct with particular emphasis to the sperm host gland were also examined.

Statistical Analysis

All the study findings were expressed as mean ± standard error (SE). Statistical analysis of the recorded data was carried out by student *t*–test using SPSS 15.0 for windows software package. When the p values were less than 0.05, the difference was considered statistically significant.

RESULTS AND DISCUSSION

The present study was carried out to observe the gross-morphometry and histomorphology of the ovary and oviduct of turkey hen in reproductive perspective with learning the distribution pattern of perm-host glands in the oviduct of turkey hen. The results and discussions of all experiments were presented below.

Gross Morphology of the Ovary and Oviduct

Ovary

The female reproductive system of the non laying turkey is composed of two parts: the ovary and the oviduct. The ovary had an irregular surface in the Coelomic cavity, cranially to the caudal extremity of the lung, ventral to the abdominal air sac, and dorsally to the kidney and adrenal gland (Figure 1). The shape of ovaries resembled a bunch of grapes consisting of numerous follicles of different sizes, which projected from the surface of the ovary and attached by a pedicle (Figure 2A). Only the left ovary and oviduct were functional in most avian species, including geese; the right ovary and oviduct, though present embryologically, degenerated during development and became vestigial in adult birds (12), but this observation was disagreement with (13, 14) who mentioned that brown kiwi and birds of prey had two functional ovaries.

Oviduct

The left oviduct was present only, and it was found in the dorsal part of the abdomen and consisted of five distinct parts: infundibulum, magnum, isthmus, uterus, and vagina; this came in accordance with (15) and (16) in domestic fowl, (17) in duck and (18) in ostrich. The oviduct was found on the caudal and dorsal sides of the abdomen and varied in size depending on the bird's reproductive activity. During the laying time, it was too voluminous, with a total length of 28-52 cm, nearly similar to the result of (19). On the other hand, (17) in Balady duck and (20), in pagan duck have recorded 45-50 cm length of the oviduct, in fowl, it reached 60-70cm (16) while (21) in rheas detected 122 cm length of left oviduct during egg-laying season. The oviduct was connected to the ovary on the rostral side, the cloaca on the caudal side, the left kidney on the dorsal side, the gizzard, and the small intestine on the ventral side, and the colon on the right side. The oviduct was kept in place by two peritoneal folds known as the dorsal and ventral oviductal ligaments. The oviduct was divided into five parts that extended from the ovary to the cloaca, including the infundibulum, magnum, isthmus, uterus, and vagina. The sperm host gland is located at the junction of the uterus and the vaginal canal and serves as a sperm reservoir. The oviduct was found in this study to be a highly convoluted structure that filled up the majority of the abdomen (Figure 1). In the experiment, the average weight, length, and width of each part of the oviduct of turkey was presented in Table 1.

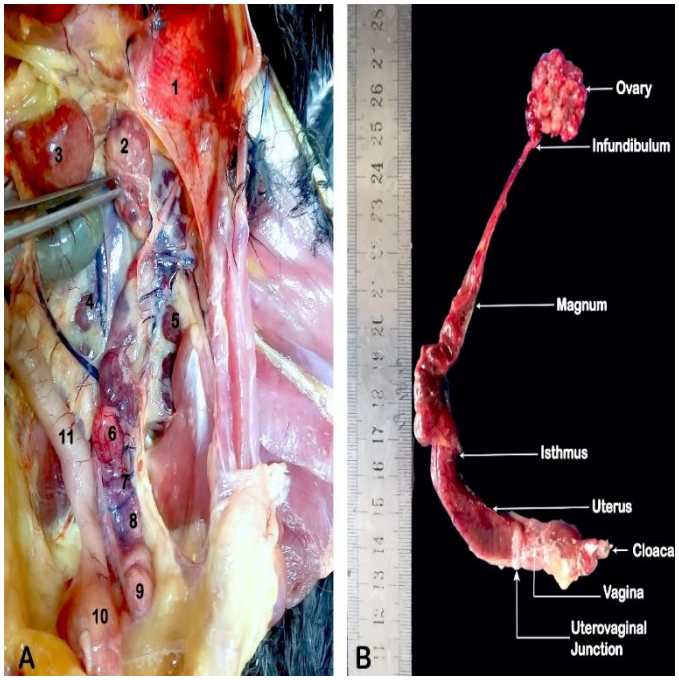

Figure 1. A) Representative photograph is showing the topography of left ovary and oviduct in non-laying turkey (ventral view): 1. Left lung; 2. Ovary; 3. Spleen; 4. Left kidney 5. Right kidney. 6. Magnum. 7. Isthmus. 8. Uterus. 9. Vagina. 10. Cloaca. 11. Colon; B) Photograph is showing the ovary and different parts of left oviduct in a non-laying turkey after dissection (the ovary and oviduct were removed outside the abdomen)

Infundibulum

The cranial ligament of the infundibulum binds the first portion of the oviduct dorsally. It involved two parts: the funnel part, flattened, thin, and translucent wall, with the opening of the fimbria facing the ovary, followed by the second part: the tubular part, which had a wall thicker than

the funnel part. The mucosal folds of the tubular portion of the infundibulum emerged in spiral ridges, which began to increase in depth towards the magnum (Figure 1 and Figure 2B). The tubular part of the infundibulum average weight was 0.23±0.15 g, length 0.97±0.11 cm, and width 0.3±0.07 cm (Table 1). The infundibulum in the current investigation consisted of a funnel and tubular parts that come in line with the findings of (17) in Balady duck, (22) in Pekin duck and (23) in Kuttanad duck; (16) in domestic fowl and (24) in ostrich.

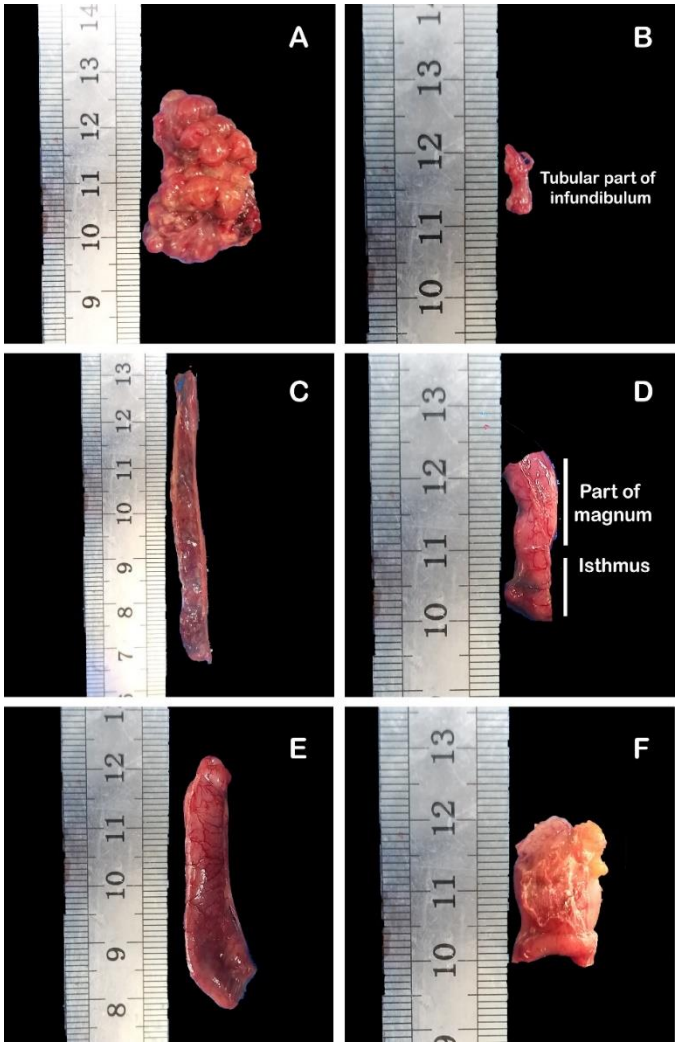

Figure 2. Representative photographs showing the (A) Ovary and different part of the oviduct of turkey hen: (B) Tubular or neck part of the Infundibulum; (C) Magnum; (D) Isthmus; (E) Uterus; (F) Vagina

Table 1. Gross morphometric observation of the weight, length, and width of different parts of the left oviduct of turkey (n=10)[1]

	Average weight (32)	Length (cm)	Width (cm)
Infundibulum	0.23±0.15	0.97±0.11	0.30±0.07
Magnum	2.74±0.16	13.6±1.26	1.61±0.25
Isthmus	0.94±0.24	1.95±0.17	0.82±0.09
Uterus/Shell Gland	3.16±0.31	8.70±1.06	1.97±0.17
Vagina	2.44±0.30	2.18±0.08	1.60±0.20

[1]Mean±standard error mean

Magnum

The second part of the oviduct appeared to be the longest and most coiled. It could be distinguished from the infundibulum morphologically by its large diameter and thick wall, as well as the presence of irregular and longitudinally arranged mucosal folds, which were distinguished by their height, especially in the cranial region, and became smaller near the isthmus, possibly due to an increase in tunica muscularis thickness or the presence of numerous tubular glands during egg production, resulting in the secretion of egg albumin (Figure 1 and Figure 2C). Its total average weight was 2.74±0.16 g, length 13.6±1.26cm, and width 1.61±0.25 cm (Table 1).

Isthmus

The isthmus was the third section of the oviduct, and it resembled the magnum in appearance but was shorter and less coiled, with a yellowish color, and its mucosal folds were smaller, smoother, and less projecting with longitudinal orientation, distinguished from the magnum by a small, light, and narrow constricted translucent area. (Figure 1 and Figure 2D). Similar results were documented in fowl and adult turkey (25), but on the contrary, in emu (26), the mucosal folds were similar to magnum. Its total average weight was 0.94±0.24 g, length 11.95±0.17 cm, and width 0.82±0.09 cm (Table 1).

Uterus or Shell Gland

The uterus, which is also known as the shell gland, was the fourth segment of the oviduct, located between the isthmus cranially and the vagina caudally. It was the thickest and widest part of the oviduct, starting as a small anterior tubular part and then expanding into a posterior pouch-like part to contain the egg during the shell-forming time. Its mucosal folds were arranged longitudinally to form leaf-like lamellae that were intersected by circular directions, which was in agreement with the observation of various authors during the histopathology of reproductive tracts of domestic fowl (27), hens (19), ducks (28) and adult turkey (29). The synsacrum, ureters, and caudal part of the left kidney were dorsolaterally related to the uterus, while the terminal part of the colon was dorsolaterally related to the uterus. The lining mucosa of the uterus had a distinct coloration in the interior, appearing granular with a greenish coloration, which could be due to the uterus glands. Furthermore, several longitudinal folds were crossed by transparent transverse corrugating grooves, while the terminal portion was devoid of both folds and grooves, allowing the vagina to continue. (Figure 1 and Figure 2E). Its total average weight was 3.16±0.31 g, length 8.70±1.06 cm, and width 1.97±0.17 cm (Table 1).

Vagina

It was the more convoluted and spiraling part of the oviduct, which was lined by whitish-yellow mucosa with about ten transverse very thick spirals intermingled folds, forming an S-shape similar to that mentioned by (30) in Malard duck but disagree with (17) who reported that mucosal folds in the balady duck were transversally arranged. These transverse folds of the vagina disappeared just 1cm before the vaginal termination. It was connected to the uterus's caudal portion cranially, the cloaca caudally, the left kidney's terminal part, and the ureter dorsally. This part did not play a significant role in egg forming, but it was important in egg-laying. It had one curvature, and its mucosal folds were arranged in a longitudinal direction (Figure 1 and Figure 2F) similar to the domestic fowls as mentioned by (31) and turkey hen (28), but two curvatures were present in the balady duck (17); and three curvatures in rhea birds (21). Its total average weight was 2.44±0.30 g, length 2.18±0.08 cm, and width 1.60±0.20 cm (Table 1).

Histomorphology of the Oviduct of Turkey

Ovary

It has been shown that the ovary of Turkey was made up of an outer cortex that surrounded an internal vascular medulla, and several ovarian follicles of various sizes inside the cortex, a germinal layer of cuboidal epithelial cells covering the cortex, a thin layer of dense connective tissue fibers forming the tunica albuginea lied below the epithelium, and stroma of loose connective tissue occurred below the tunica albuginea. Ovarian follicles with different stages of development occurred throughout the stroma of the cortex that classified into primordial, primary, secondary, and tertiary follicles, as well as the presence of Corpus Luteum (13) connective tissue with remnants of blood clots that were gradually removed (Figure 3A) these were in consonance with the finding of (33) in fowl; (34) in Duck.

The present study showed that the ovary of turkey was consist of an outer cortex which enveloped an internal vascular medulla and numerous ovarian follicles of different sizes present within the cortex, similar description reported by (35) in chicken but disagreement with (34) in duck who mentioned that ovary consisted of simple squamous epithelium with patches of cuboidal epithelium. The primordial follicle can be distinguished from the primary follicle by its size; the primary follicle had a greater volume and a homogeneous cytoplasm of small granules than the primordial follicle. The secondary follicle was found in the middle of the cortex and was characterized by larger follicles that contained well-developed oocytes with clear cytoplasm and fine granules with a central or eccentric nucleus. In contrast, the tertiary and mature follicles contained growing oocytes that were covered by many layers: the theca externa, theca interna, membrane granulosa, and privitelline membrane.

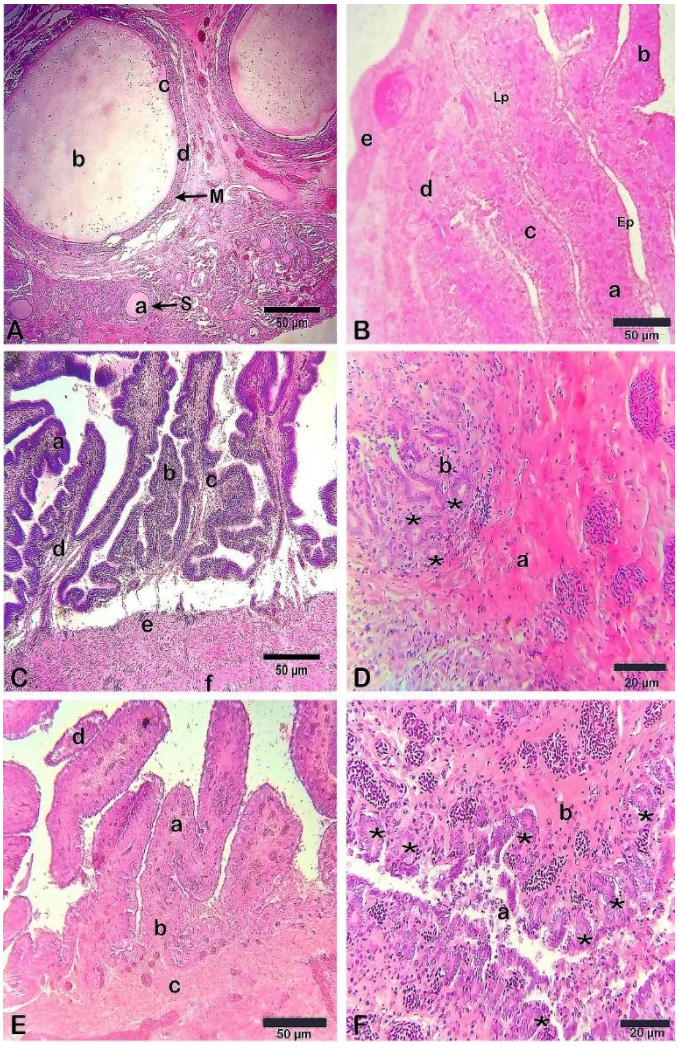

Figure 3. (A) Hematoxylin and Eosin stained histological section in the ovary showed: S- secondary follicle M- mature follicle a- Oocyte b- Growing Oocyte c- granulosa cells d- Theca follicular cells (Scale Bar=50 µm); (B) Histological section in tubular part of infundibulum shows: a- primary fold, b- secondary fold, c- lamina propria, d- muscularis mucosa e- tunica serosa, Ep- Luminal epithelium, Lp- Lamina propria (Scale Bar=50 µm); (C) Histological section in magnum shows: a- primary fold, b- secondary fold, c- lamina propria, d- inner muscularis mucosa, e- outer muscularis mucosa, f- serosa (Scale Bar = 50 µm); (D) Histological section in isthmus shows: a- lamina propria, b- sub mucosa filled with branched tubular glands (stars) (Scale Bar=20 µm); (E) Histological section in uterus shows: a- leaf-like mucosal folds, b- lamina propria, c- inner muscularis mucosa with d- blood vessels (Scale Bar=50 µm); (F) uterus shows: a- submucosa filled with branched tubular glands, b- lamina propria (Scale Bar=20 µm)

The theca externa was made up of dense connective tissue that included clusters of pale interstitials (luteal) cells that might also present in the cortical stroma and medulla in classes. The description of primordial, primary, secondary, tertiary, mature follicles, and theca externa were in agreement with that previously mentioned by (13, 36) in the pigeon; (37) in chicken.

Oviduct

The wall of the oviduct in the indigenous geese consisted of several tunicae which arranged from the internal surface

to the external surface, respectively named: tunica mucosa, tunica submucosa, tunica muscularis, and tunica serosa.

Infundibulum

It was divided into the funnel and tubular parts. Long finger-like projections were attached to the funnel portion that represented the fimbria, which were lined with pseudo stratified columnar ciliated epithelium similar to that reported by (38) in guinea fowl although (39) in turkey hen mentioned that the epithelium of the infundibulum was variable from simple cuboidal in the upper part into ciliated simple columnar in the middle and lower end. The funnel part's lamina propria was composed of loose connective tissue that was densely packed with blood vessels but absent of tubular glands (Figure 3B).

Magnum

It was the longest part of the oviduct that had well-developed tubular glands that produced the albumin. The mucosal folds were numerous and variable in size than those of the infundibulum, branched into primary and secondary folds, lined with pseudo stratified columnar ciliated secretory epithelium, the lamina propria-submucosa contained branched tubular glands, and the tunica muscularis-mucosa contained inner circular and outer longitudinal smooth muscle layers (Figure 3C); these results were in parallel to that mentioned by (37) in birds, (40) in ostrich; (22) in turkey and pigeon.

Isthmus

its mucosal folds were arranged longitudinally, branched into primary and secondary folds, lined with pseudo stratified columnar ciliated epithelium. The lamina propria-submucosa contained branched tubular glands similar to those of magnum but shorter and fewer that secrete shell membranes, tunica muscularis better developed than the magnum's and composed of thick inner circular and thin outer longitudinal (Figure 3D) and these information were declared previously by (31) in the domestic fowls; (37) in chicken; (21) in rhea birds.

Uterus

Its mucosal fold was wider, smaller, and branched into primary and secondary looked like leaf-like lamellae that were lined with pseudo stratified columnar ciliated secretory epithelium. The lamina propria-submucosa had loose and well-vascularized connective tissue spared between the branched tubular glands; the tunica muscularis was thicker and better vascularized; and the tunica muscularis was made up of thin outer longitudinal and comparatively thicker inner circular smooth muscles (Figure 3E-F) in comparison to the other parts similar findings reported by (41) in the laying hen and duck; (42) in the Japanese quail, (42) in the Japanese quail; (37) in birds.

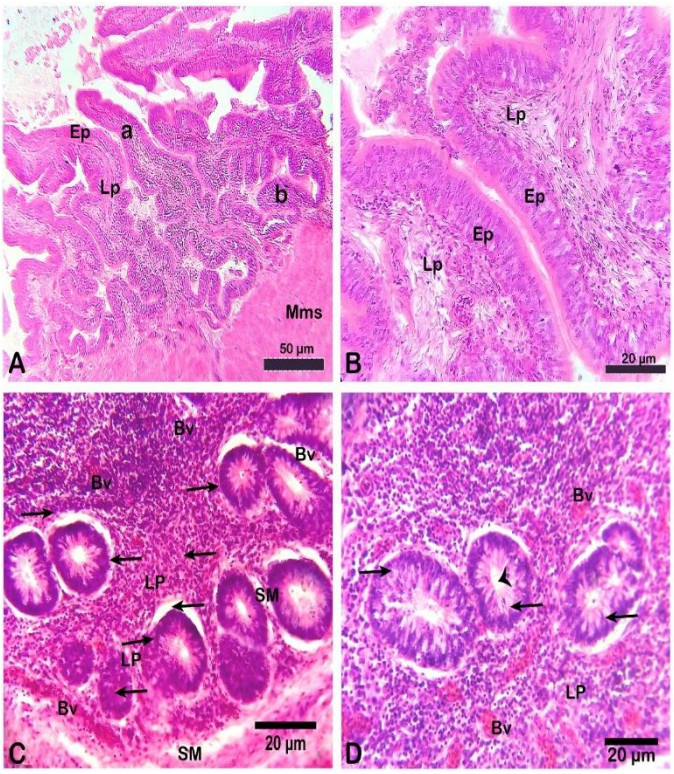

Figure 4. (A) Hematoxylin and Eosin-stained histological section in vagina shows: a-primary fold, b- secondary fold, Ep- Luminal epithelium, Lp- Lamina propria with Mms- Muscularis mucosa layer (Scale Bar=50 µm); (B) Histological section in vagina shows: pseudo stratified columnar ciliated epithelium in the Luminal epithelium (Ep), Lp- Lamina propria (Scale Bar=20 µm); (C) Histological section of uterovaginal junction (UVJ) of turkey shows increase in number of sperm-storage tubules (SST) or sperm host glands (SHG) (arrow) and different cell types in UVJ. In the UVJ, the SST is distributed in the lamina propria of mucosal folds. SST (arrows) are lined by simple columnar epithelium and observed in the lamina propria-submucosa. (Scale Bar=20µm). SM-Sub mucosa, LP-Lamina propria, Bv-blood vessels; (D) Histological section infundibulo-magnum junction of turkey shows sperm host glands (SST) and different cell types. The SST consists of a single layer of non-ciliated epithelial cells. No sperm is observed in the lumen of SST. (H&E stain, Scale Bar=20 µm). LP-Lamina propria, Bv- blood vessels. (Scale Bar=20 µm)

Vagina

It was a short and narrow part. Its mucosa was arranged as numerous tall and narrow primary folds that carrying small secondary folds, lined by pseudo stratified columnar ciliated epithelium. The lamina propria-sub mucosa was lack of tubular glands. The tunica muscularis was well developed particularly the circular layer which represented the thickest part of the vaginal wall forming the vaginal sphincter, as well as, the outer longitudinal smooth muscle fibers, serosa made of loose connective tissue (Figure 4A-B) similar results also found by (21) in rhea birds; (37) in chicken.

Histology of Sperm-Host Gland (SHG)

The utero vaginal junction (UVJ) of the oviduct was lined by pseudostratified columnar type epithelium containing ciliated, nonpiliated, goblet and basal cells. Sperm-storage tubules were observed in the uterovaginal junction and

these tubules were mostly branched and slightly coiled and extended into the lamina propria from the bases of the mucosal folds. They were somewhat more sparsely distributed and less convoluted than the uterine glands, though larger in diameter. Each tubule was lined with a columnar epithelium that rested on a basal lamina and was close to blood vessels. The basal nuclei of these glands were darkly stained, while the cytoplasm was finely stained (Figure 4C-D). Some lightly stained glands in between the proprial glands with narrow lumens and morphologically similar to sperm host glands were also found at the infundibulo-magnal junction. Similar type of sperm host glands was also observed at the infundibulo-magnum junction of domestic quail and their number was higher than in uterovaginal junction (43). The number of sperm host glands was more at uterovaginal junction than infundibulo-magnal junction (Figure 4 C-D) in the present study as reported earlier by (44). Similar findings were reported by (45) at uterovaginal junction in ostrich, in hen by (46, 47). These glands had proximal and distal parts; the proximal part was lined by pseudostratified columnar epithelium and distal part by non-ciliated simple columnar epithelium (Figure 3). The proximal part was considered as neck region whereas distal part as proper sperm hosts glands. These findings corroborated well with the observation of (48) in American Kestrel, (49) in Japanese quail and (50) in Punjab white quail. The sperm-host glands were specialized tubular infoldings of the chicken infundibulum and vagina. They were involved in the storage and survival of spermatozoa in the oviduct (51) so that chicken could produce fertile eggs up to several weeks after a single mating or artificial insemination.

The oviduct is an extremely convoluted and muscular structure that makes up the whole female genital tract and undergoes a number of changes during egg formation. The oviduct is of particular importance to the commercial egg industry because any oviduct malfunction may have a significant impact on the eggshell quality. Infundibulum, magnum, isthmus, uterus, and vagina were the five regions of the oviduct. While much study has been done on the oviduct in chickens and other poultry species, there is a scarcity of detailed histomorphological data on the oviduct in turkeys. The current study provides an excellent explanation of the various parts of the oviduct, especially the distribution of the sperm host gland in the turkey oviduct, which may play a functional role in the storing and release of spermatozoa from the SHG in response to oviposition or ovulation. The value of studying the oviduct in poultry merges from a financial point of view in order to produce the most products, such as fertilized eggs from the poultry.Interestingly, observations of current study may help poultry scientists and farmers in formulating successful development and disease control strategies.

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AUTHOR CONTRIBUTIONS

MRI designed the experiment. IH performed the experiments. IH analyzed the data and wrote the draft. MRI, IH, NZM and MA critically review, editing and revised the manuscript.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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