

Gross and Microanatomical Studies on the Uterus of Japanese Quail (*Coturnix japonica*) During the Post-hatching Period with Special Emphasis on Sperm Host Gland

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Abstract

The quail acts as a model for recent experimental studies and its oviduct development is of special interest. This study was carried out on 61 adult female Japanese Quail (*Coturnix japonica*) collected from quail farms in Assiut and South Valley Universities in order to characterize the morphological features of the uterus during the post-hatching life. The process of development was consisted of three stages: undifferentiated, differentiated and adult stage. The undifferentiated stage began from day of hatching till 25-days old age and the oviduct was divided into cranial, middle and caudal parts. The caudal part (the future uterus and vagina) was represented by a simple wall of a single layer of simple columnar epithelial cells resting on subepithelial undifferentiated mesenchymal cells with many mitotic divisions. At 20 days old, the mucosa is thrown into distinct mucosal folds as well as the ciliated and secretory epithelial cells were first appeared at this age. At differentiated stage (from 30-40 days), the tubular glands began to develop and opened between the epithelial cells. The adult stage began from 50 days old and the luminal surface of the uterus was characterized by numerous long leaf-like folds. Also, the apical part of the cells showed positive reaction with PAS and Alcian blue. The tubular glands are filled with the secretion, which give positive reaction with PAS and Alcian blue. The uterovaginal junction lies between the uterus and the cranial part of the vagina, and contains the sperm host gland. This area showed transition of the mucosal folds from longitudinal folds of the uterus to complex interconnecting folds of the vagina. Many typical sperms with oval head and tail were inserted between the ciliated and microvillous surface epithelial cells of sperm host glands. This detailed anatomical and histological study of post-hatching development of uterus in quail will not only help to evaluate changes occurs during this critical period, but also will assist in understanding clearly the physiology of reproduction in quail.

Keywords: Uterus; Sperm host gland; Post-hatching development; Quail

Introduction

The oviduct is a complex biological organ that undergoes a series of hormonal, neuronal, biochemical and cellular changes during formation of an egg. This duct is concerned with the transport of the ovum away from the ovary, secretion of the shell membrane, fertilization of the ovum as well as deposition of albumen, membranes and shell on to the ovum to form the finished egg [1].

In general, the oviduct of adult bird is well developed at the left side, atrophied at the right side and consisted of infundibulum, magnum, isthmus, uterus and vagina [2,3]. While in early ages, it can be divided only into cranial, middle and caudal parts. Many studies have been done in order to describe the characteristic morphological features of the oviduct in many avian species including fowl [4], duck [5], turkey [1] and ostrich [6].

The epithelium lining the oviduct in birds is consisted mainly of ciliated and non-ciliated secretory cells. The oviductal epithelial cells show marked regional variations in anatomical, histological and histochemical structures in many species [7,8].

Bakst [9] found that the sperm storage sites are either primary or secondary. The uterovaginal junction is the primary sperm storage sites in the oviduct that constituted mainly of sperm-storage tubules, which

is a narrow band at the cranial end of the vagina. These specialized subepithelial tubular glands are capable of only limited secretory activity. The chalaziferous region, which referred to the distal infundibulum acts as the secondary sperm-storage site in the oviduct [10]. It is characterized by sub epithelial tubular glands, which secrete an albumen-like material, which provides an additional fibrous protein investment around the ovum and prevents excessive sperm penetration of the ovum at the cranial end of the oviduct at the process of fertilization [11].

Japanese quail (*Coturnix japonica*) is widely distributed in East Asia; includes India, Korea, Japan, and China [12,13]. This species has also been found to reside in many parts of Africa extending from Kenya to Egypt. It is characterized by easily managing, fast growing, high- protein food source, and can produce eggs at high rate [14]. Recently, Japanese quail acts as a model for many experimental and biomedical researches. It is now widely used in many Fields include embryology, genetics, nutrition, physiology, pathology, cancer, behavior and the toxicity of pesticides [15].

The aim of the present study is to describe the gross morphology, histomorphometry, histochemistry as well as surface architecture of the uterus during the post-hatching life with special reference to the sperm host gland of Japanese quail (*Coturnix japonica*). These investigations will contributed to better understanding of the significance of these structures in physiological and ecological aspects of bird reproduction.

Materials and Methods

Sample collection:

This study was carried out on 60 female Japanese Quail collected from quail farms in Assiut University (Faculty of Agriculture) and South Valley University (Faculty of Science). The specimens under examination were collected from day of hatching, 5, 10, 15, 20, 25, 30, 35, 40, 50 days, 4 months and 6 months old (5 birds from each age). The birds before 40 days were anesthetized by chloroform in a suitable box before slaughtering. While adult birds were anesthetized with xylazine-ketamine combinations at the dose rate of 2 mg xylazine and 10 mg ketamine per kg that injected to breast muscles.

Gross morphological examination

The body cavity of each bird was opened and the body wall was reflected in both sides. The topography of the viscera and other organs was photographed by a digital camera. The position and relations of the uterus to the neighboring organs were recorded. Also, the ligaments of the uterus were examined concerning the origin and termination and photographed. Gross morphology of the uterus during the post-hatching period was described. For the fine arterial vasculature of the uterus, serum Indian ink was injected in four anesthetized adult birds through the left ventricle of the heart. The injected oviducts were examined after passing ascending grades of alcohols (50: 100%), then methyl benzoate. Hand free sections were examined in addition to paraffin slides (10 & 20 microns) and then photographed.

Histological examination:

Histological analysis: Specimens were dissected as soon as possible from uterus from day of hatching till 6 months old age. Also specimens from uterovaginal junction of the adult birds were dissected at $1 \times 1 \times 0.5$ cm and were immediately fixed in Bouin's solution. The fixed samples were dehydrated, cleared, embedded in paraffin wax, sectioned at thickness of 4 μ m and stained with Harris's Haematoxylin and Eosin, Crossmon's Trichrome and Wiger's Elastica [16].

For semithin sections: Representative specimens at day of hatching, 10, 20, 40, 50 days and 4 months were used for semithin sections. Small pieces 2.0-3.0 mm long from uterus were placed on 2.5% cold glutaraldehyde in phosphate buffer (PH 7.2) for 24 hrs. The pieces were washed twice in 0.1 M phosphate buffer and then post-fixed in 1% osmium tetroxide, in the same buffer. The post-fixed pieces were dehydrated in graded alcohols and embedded in araldite resin. Semithin sections (1 μ m) in thickness will be stained with 1% toluidine blue.

Histochemical analysis: Periodic Acid-Schiff (PAS) technique for demonstration of neutral mucopolysaccharides and Alcian blue technique (pH 2.5) for demonstration of acid mucopolysaccharides were used at all developmental stages [16].

Scanning electron microscopy: Specimens from uterus at 10, 30, 50 days and 4 months post-hatching period were taken with specimens from the uterovaginal junction of adult quails. The samples were washed for several times in normal saline and acetic acid 2% then fixed in 4% glutaraldehyde solution for 24 hrs then post fixed in 2% buffered osmium tetroxide. The fixed samples were washed in 0.1 M cacodylate buffer at PH 7.3 then dehydrated in ascending grades of ethanol, critical point dried in liquid carbon dioxide, and mounted on metal stubs then coated with gold palladium in sputtering device.

Specimens were examined and photographed by JEOL scanning electron microscope (JSM-5400 LV) operated at KV 10.

Morphometrical and statistical analysis: Morphometrical measurements to uterus at different post-hatching ages were performed by using image analysis tools (IT system). These measurements include:

- a- The diameter of uterus and the thickness of its walls (μ m).
- b- The number of mucosal folds /cross section.
- c- The height of primary mucosal folds (μ m).
- d- The width of primary mucosal folds (μ m).
- e- The height of the surface epithelium (μ m).
- f- The thickness of the muscular layer (μ m).

All the respective data were expressed by mean \pm standard error. The anatomical terms used in this study were those of the Nomina Anatomica Avium (1979).

Results

Gross morphology

The process of development of the oviduct in quail during the post-hatching period was consisted of 3 stages; undifferentiated, differentiated and adult stages.

Undifferentiated stage: The oviduct at the day of hatching till 5 day old was related dorsally to the left kidney, laterally to the lateral body wall and ventrally to the intestine (Figure 1A). The oviduct was transparent and nearly had the same diameter along its length. At this stage, the oviduct could be divided into cranial, middle and caudal parts. The caudal part of the oviduct (represented the uterus and vagina of adult bird) at 10-15 days extended caudally till its termination in the urodeum (Figure 1B) and it slightly enlarged than the cranial and middle parts and appeared black in color due to the concentration of dark pigment in its wall (Figure 1C). The oviduct at 20-25 days old enlarged in both thickness and diameter. It showed the first indication of the process of differentiation into its different parts (Figure 1D).

Differentiated stage: At 30 days old, the caudal part became greatly enlarged in diameter and continued caudally forming the uterus and vagina (Figure 2A). At 35 days old, the oviduct was highly enlarged and was completely differentiated into its final five parts. The uterus developed into a large sac like dilatation, which was attached to the other parts of the oviduct by the dorsal and ventral ligaments (Figure 2B). At 40 days old, the whole oviduct became well-developed and enlarged both in width and length to be ready for receiving the ovulated ovum. At this age, the uterus represented the dilated part of the oviduct and its wall was encircled externally by 6-7 grooves, which were demarcated 7-8 transverse ridges. These grooves formed the routes for the passage of uterine blood vessels (Figure 2C).

Adult stage: The oviduct of the adult laying quails (aged 50 days, 4 and 6 months) was found in the left half of the body cavity extending from the left ovary till its termination in the urodeum of the cloaca. It lied medial to the left body wall and dorsal to the coils of the intestine. The uterus of the laying birds was large in size with wide lumen and darkly pigmented wall. Its shape was sac-like and occupying the

caudal quarter of the left body cavity with slight inclination to the right (Figure 3A).

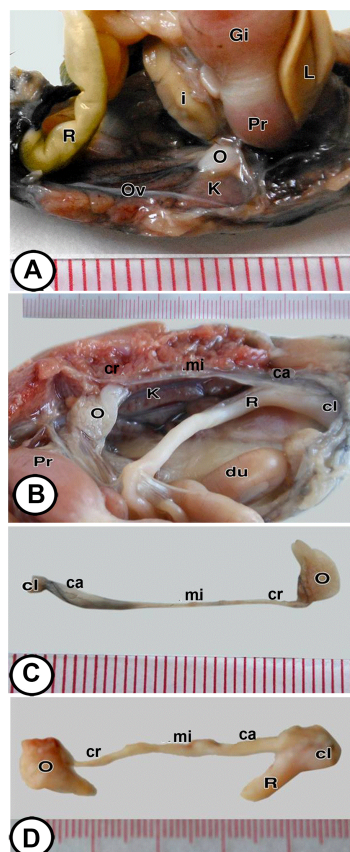


Figure 1: Gross morphology of the oviduct at the undifferentiated stage. **Figure 1A:** The undifferentiated left oviduct (Ov) of quail at day of hatching lies ventral to the left kidney (K). It appears apart and lateral to the left ovary (O). Its average length is about 1.5 cm. Gi: Gizzard; i: Intestine; L: Liver; Pr: Proventriculus; R: Rectum. **Figure 1B:** The oviduct at 10-day extends lateral to the left ovary (O) till the cloaca (cl) and ventral to the left kidney (K). It is divided into three parts; cranial (cr), middle (mi) and caudal (ca) parts. du: Duodenum; Pr: Proventriculus; R: Rectum. **Figure 1C:** The duct at 10- days is about 2.7 cm in length. The caudal part (ca) appears slightly enlarged and darker in color. cl: Cloaca; O: Ovary. **Figure 1D:** Showing more developed parts of the oviduct at 20- days. The duct is about 4 cm in length. cl: Cloaca; O: Ovary; R: Rectum.

For examination the fine arterial vasculature of the wall of the uterus, serum Indian ink was injected in the left ventricle of the heart, and reached all the arterial blood vessels of the bird, and the organs appeared black in color (Figure 3B). In the uterus, the injected specimens showed high longitudinal mucosal folds with secondary short ones, each was supplied by a central arteriole. This gave also collateral branches, which ascend toward the apex forming continuous collateral vessels. The uterine glands appear concentrated at the periphery of the folds and were richly supplied with blood (Figures 3C and 3D).

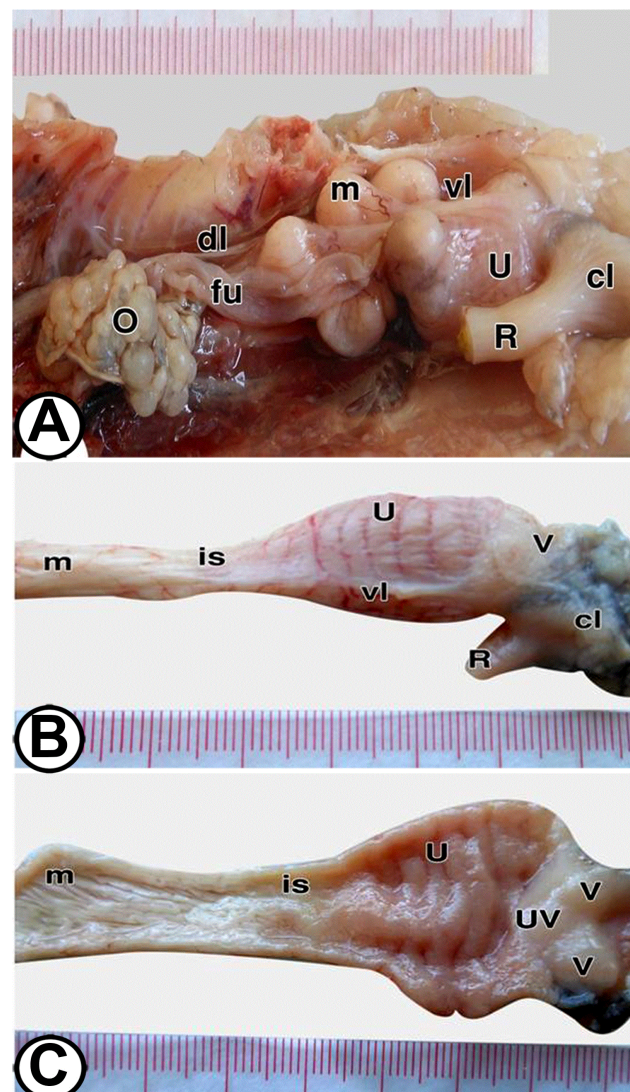


Figure 2: Gross morphology of the caudal part of the oviduct at the differentiated stage: **Figure 2A:** at 30-days old, the ovary (O) showing moderately developed follicles, and the oviduct differentiated into infundibulum with its distinct funnel (fu), magnum (m) with many coils that affected by dorsal and ventral ligaments (dl, vl) and uterus (U) that showing oblique ridges externally. cl: Cloaca; R: Rectum. **Figure 2B:** Higher magnification of the caudal part of the oviduct at 35 days, showing the transverse ridges on the wall of the uterus (U), and the termination of the ventral ligament of the oviduct (vl). Note the junction of the uterus with vagina (v) and isthmus (is) that connected with magnum (m). cl: Cloaca; R: Rectum. **Figure 2C:** Opened caudal part of the oviduct at 40- days showing spirally twisted folds of the magnum (m), short folds of the isthmus (is), transverse oriented ridges of opened uterus (U), utrovaginal junction (UV) and vagina (V) that showing vaginal sphincter.

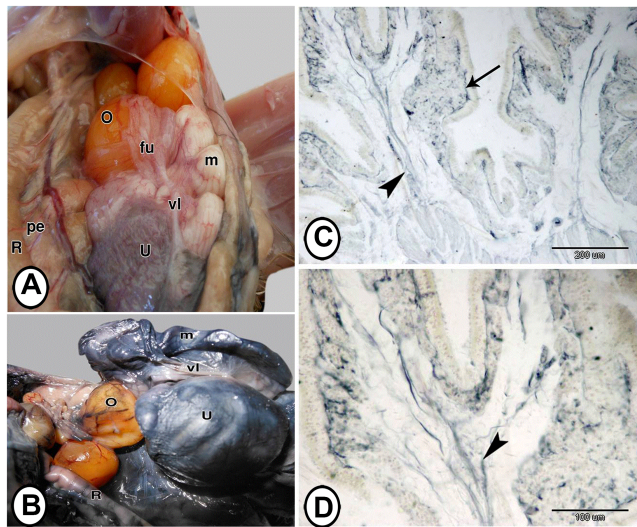


Figure 3: Gross morphology of the uterus at adult stage and quail samples injected by serum Indian ink. **Figure 3A:** adult stage showing large mature ovum (O), which is engulfed by funnel part of infundibulum (fu), coils of magnum (m), large darkly pigmented uterus (U) and ventral ligament of the oviduct (vl) attached to the infundibulum, magnum and uterus. Pe: peritoneum; R: Rectum. **Figure 3B:** Adult quail injected with serum Indian ink showing ovary (O) with blood vessels filled with stain, magnum (m) with stained blood vessels in its wall. Uterus (U) contains an egg and its wall is stained black by serum Indian ink. R: Rectum; vl: Ventral ligament of the oviduct. **Figures 3C and 3D:** histological specimens injected by serum Indian ink showing peripheral arterial branch (arrow), which supplied clusters of uterine glands. Central arteriole (arrowheads) was found in the bulbs of the primary and secondary folds.

Histological analysis

Undifferentiated stage: At day of hatching till 5 days old, the three parts of the oviduct had a similar general structure; the lumen was narrow and their walls were represented by a single layer of simple columnar epithelial cells with large vesicular nuclei rested on subepithelial connective tissue that made up of densely packed fusiform undifferentiated mesenchymal cells (Figures 4A and 4B). At 10-15 days, the lumen of the caudal part appeared wider with many short tongue-shaped mucosal folds (Figure 4C). The mucosal folds were lined by simple columnar epithelium with many mitotic divisions (Figure 4D). The lamina propria- submucosa was represented by a layer of connective tissue containing mesenchymal cells, which arranged in a regular manner to form the future tubular glands (Figure 4D).

At 20-25 days, the mucosa of the caudal part was thrown into distinct short mucosal folds (Figure 4E). Semithin sections revealed appearance of ciliated and secretory cells in the epithelial lining of the mucosal folds for the first time at this age (Figure 4F). The epithelium showed no secretory activity to PAS (Figure 4G). These cells were resting on a highly cellular connective tissue layer containing collagenous fibers and covered from outside by flatten cells of the peritoneal serous membrane (Figure 4H). The tunica muscularis began

to develop and was consisted of isolated smooth muscle fibers arranged in a circular manner (Figure 4F).

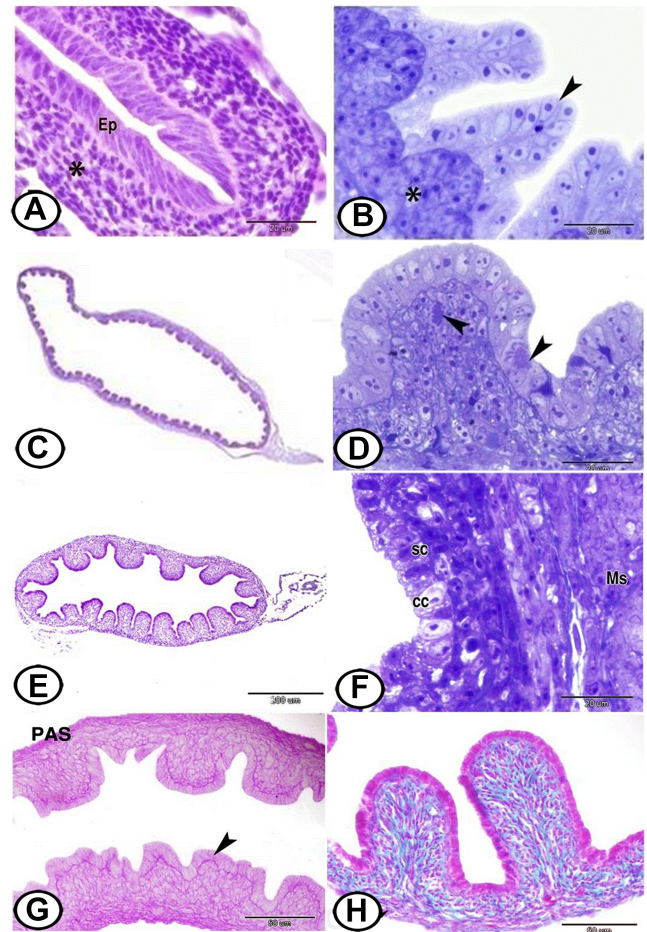


Figure 4: Histological, histochemical analysis of the undifferentiated stage. **Figure 4A:** The wall of the caudal portion of the oviduct at day of hatching formed of simple columnar epithelium (Ep), rested on undifferentiated tunics (asterisk). (H & E). **Figure 4B:** Semithin section of the caudal portion at the day of hatching showing simple columnar epithelium (arrowhead) rested on undifferentiated mesenchymal stroma (asterisk). (TB). **Figure 4C:** General view of caudal portion at 10- days showing numerous short mucosal folds. (H & E). **Figure 4D:** In semithin section, the epithelium at 10- days is formed of simple columnar cells, with many mitotic divisions in the epithelium and stroma (arrowheads). (TB). **Figure 4E:** General view of the caudal portion of the oviduct at 20-days old. (H & E). **Figure 4F:** In semithin section of the caudal portion at 20-days old, the epithelium consisted of ciliated (cc) and secretory cells (sc). The muscle layer differentiated into inner circular and outer longitudinal smooth muscle fibers (Ms). (TB). **Figure 4G:** The epithelium at 20-days showing negative reaction by PAS (arrowhead). **Figure 4H:** Crossmon's Trichrome showing abundant collagenous fibers, which were extended into the folds.

The morphometrical analysis of the undifferentiated stage is represented in (Table 1).

Age	External diameter (μm)	Thickness of wall (μm)	Number of folds / cross section	Height of primary fold (μm)	Width of primary fold (μm)	Height of epithelium (μm)
Day of hatching	139.54 ± 4.1	29.99 ± 2.5	13.0 ± 2.8	14.04 ± 1.5	26.16 ± 1.2	7.10 ± 0.3
5 days	316.3 ± 5.1	66.37 ± 2.9	22.5 ± 0.8	27.47 ± 1.4	32.61 ± 2.0	7.86 ± 0.14
10 days	411.68 ± 3.8	67.44 ± 2.6	30.1 ± 0.76	22.41 ± 1.7	35.75 ± 1.9	6.06 ± 0.18
15 days	574.52 ± 4.9	90.2 ± 3.1	33.4 ± 0.21	55.20 ± 1.5	47.93 ± 1.1	6.54 ± 0.32
20 days	691.60 ± 6.0	97.56 ± 3.8	21.0 ± 0.84	64.83 ± 2.0	56.54 ± 2.3	7.06 ± 0.22
25 days	1149.99 ± 5.4	164.89 ± 4.3	42.7 ± 0.49	84.04 ± 1.6	82.86 ± 3.1	11.16 ± 0.46

Table 1: The morphometric values of the caudal part of the oviduct at undifferentiated stage.

Differentiated stage: At 30-35 days old age, the left oviduct became more differentiated into its tunics but still subdivided into cranial, middle and caudal parts. The expanded caudal part, which represented the uterus, was well-recognized due to the greater diameter than the other parts. The mucosal folds were markedly increased in height (Figure 5A and Table 2). The lamina epithelialis was formed of ciliated and non-ciliated secretory cells (Figure 5B). The epithelial cells showed negative reaction with PAS (Figure 5C). However weak positive reaction with Alcian blue stain at the apical part of the epithelium was recorded (Figure 5D). In the lamina propria-submucosa, the mesenchymal cells differentiated into tubular glands. At the base

between each two neighboring folds, the tubular glands began to develop and opened between the epithelial cells (Figure 5B). Abundant network of elastic fibers were demonstrated in lamina propria-submucosa and around blood vessels (Figure 5E). At 40-days, the semithin sections showed that ciliated cells were characterized by its large size and pale staining cytoplasm with enlarged rounded to ovoid nucleus located centrally and provided with long cilia. Moreover, the secretory cells appeared narrow slender dark cells with basal nucleus. The glands were constituted of groups of short cells with rounded nucleus opened by their ducts to the epithelial surface (Figure 5F).

Age		External diameter (μm)	Thickness of wall (μm)	Number of folds /cross section	Height primary of fold (μm)	Width primary of fold (μm)	Height epithelium (μm) of	Thickness of muscular layer (μm)
30 days	Caudal part	2014.28 ± 6.9	301.37 ± 2.8	60.9 ± 2.3	139.11 ± 1.3	91.86 ± 1.4	11.52 ± 0.7	107.68 ± 1.4
40 days	Uterus	9344.19 ± 7.2	4190.48 ± 5.7	83.1 ± 2.1	3500.36 ± 5.8	272.81 ± 2.4	23.63 ± 0.65	415.29 ± 1.7

Table 2: The morphometric values of the caudal part (uterus) of oviduct at differentiated stage.

The morphometrical analysis of the differentiated stage is represented in (Table 2).

Adult stage

From 50 days to 6 months, the luminal surface of the uterus showed numerous long leaf-like folds, which were branched into primary and secondary ones (Figure 6A). These mucosal folds were protruded into the lumen and became expanded at its apical part forming an amputated structure (Figure 6B).

In semithin section the epithelium was consisted of large light ciliated cells and secretory cells that filled with metachromatic secretory granules (Figure 6C). The lamina propria was loosely packed with branched tubular glands, which were rounded to oval in shape, formed of simple cuboidal cells with centrally located nucleus. The secretory cells of these glands were typically vacuolated and granulated and showed weak metachromasia (Figure 6D). The apical borders of the epithelium showed strong positive reaction to PAS and Alcian blue. Also, the lumen of glands was filled with the secretion, which gave positive reaction with PAS and Alcian blue staining (Figures 6E and 6F). The lamina propria-submucosa was consisted of loose and highly vascularized connective tissue that formed mainly of collagenous fibers (Figure 6G) and abundant elastic fibers, which present between the

muscle bundles, within the folds and around the blood vessels in the muscular layer (Figure 6H). The Tunica muscularis was consisted of distinct inner circular and outer longitudinal smooth muscle fibers which were separated by a layer of highly vascularized vessels of different sizes (Figure 6H). The Tunica serosa was composed of loose connective tissue layer covered by single layer of flat mesothelial cells (Figure 6G).

The morphometrical analysis of the uterus at the adult stage is represented in (Table 3).

Scanning electron microscopy

At 10 days-old, the mucosa of the caudal portion of the oviduct showed a series of unbranched longitudinal aligned folds, which were separated from each other by narrow slits (Figure 7A). Single cilium was observed at higher magnification projecting from the surface among the epithelial cells (Figure 7B). The uterus at 30-days showed wavy parallel longitudinal mucosal folds, which were separated by deep furrows (Figure 7C). Numerous blebs- like secretions were accumulated on the surface indicating apocrine mode of secretion. Patches of long cilia (2 μm) were scattered randomly between non-ciliated cells. The non ciliated cells were covered by distinct microvilli (Figures 7D and 7E).

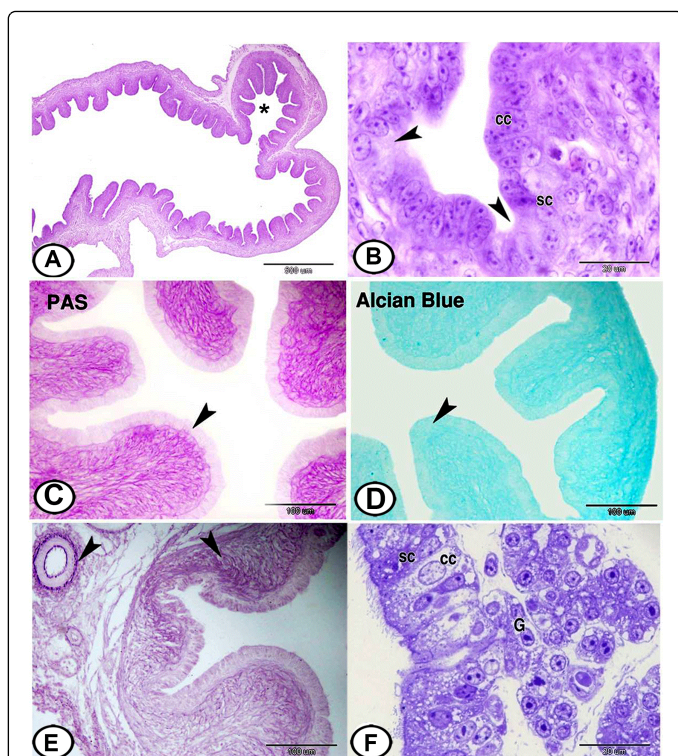


Figure 5: Histological and histochemical analysis of the differentiated stage (30-40 days). **Figure 5A:** Cross section in the caudal part of the oviduct at 30-days old showing wide lumen and numerous folds. Note the beginning of differentiation of sperm host gland (asterisk). (H & E). **Figure 5B:** Higher magnification showing the ciliated (cc) and secretory (sc) lining epithelium with beginning of formation of glands at the base of the fold (arrowheads). (H & E). **Figure 5C:** The epithelium showing negative reaction by PAS (arrowhead). **Figure 5D:** Alcian blue gave weak positive reaction at the apical portion of the epithelium (arrowhead). **Figure 5E:** Many elastic fibers which present within the folds and around the blood vessels (arrowheads). (Wighert's Elastica). **Figure 5F:** In Semithin section at 40-days old, the epithelium showing ciliated (cc) and secretory cells (sc). Note presence of glands (G) that opened to the epithelium by their ducts. (TB).

The mucosa of the uterus of adult Japanese quail showed numerous complex mucosal folds with many clefts between them (Figure 8A). The surface was covered by densely packed ciliated cells enclosing mosaic-like pattern arrangement of non-ciliated cells with apical microvilli (Figure 8B). Many slit like openings for glands were demonstrated in the luminal surfaces (Figure 8C). The mucosa was covered by numerous ciliated cells with few microvillous cells in between them provided with small bleb-like secretions (Figure 8D).

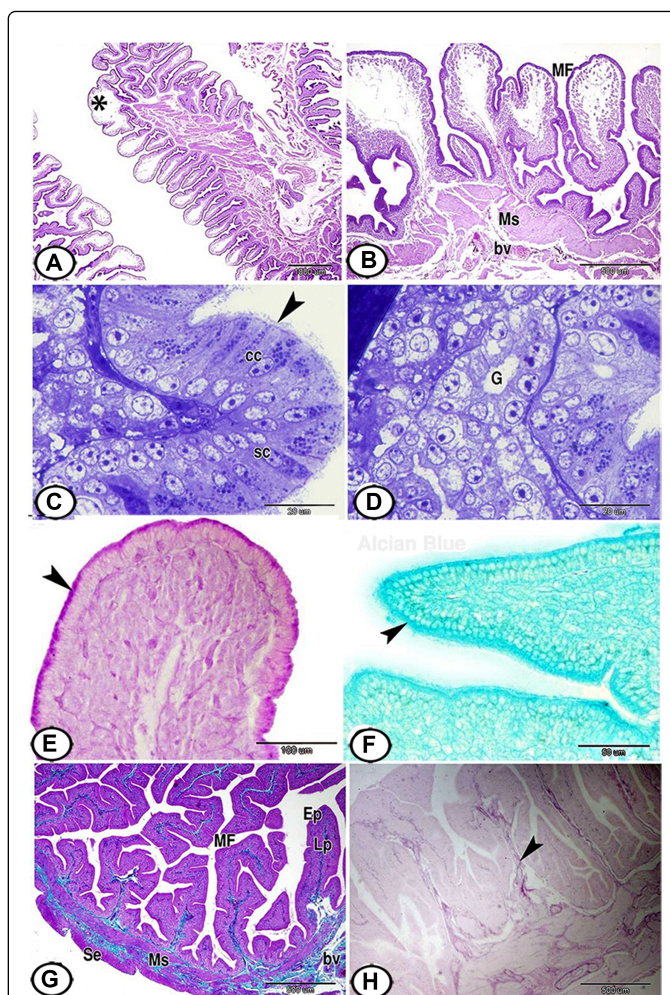
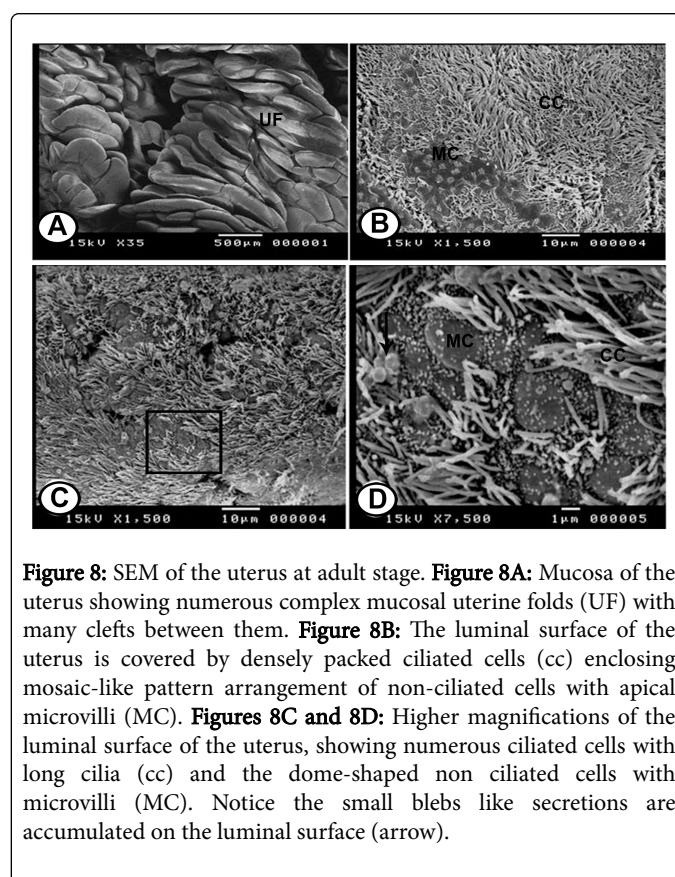
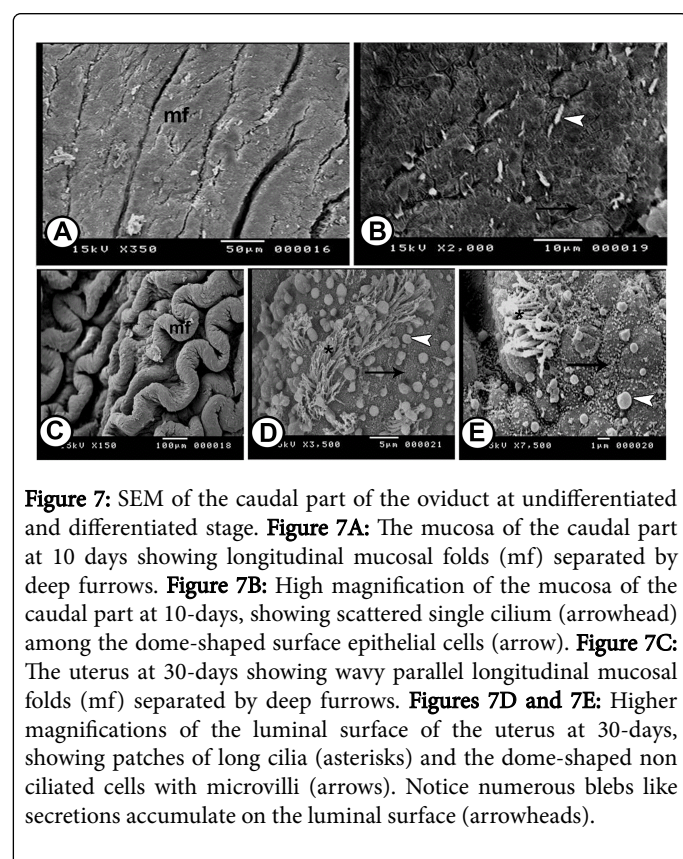


Figure 6: Histological and histochemical analysis of uterus at adult stage. **Figure 6A:** Wall of the uterus showing the expanded apices of long folds (asterisk) (H & E). **Figure 6B:** Higher magnification of the wall of uterus showing expanded mucosal folds (MF) & thick muscular layer (Ms) with large blood vessels (Bv) (H & E). **Figures 6C and 6D:** Semithin sections of mucosal fold show the epithelium that consists of ciliated cells (cc) with long cilia (arrowhead) and secretory cells (sc) with metachromatic granules. Note, presence of glands (G) underlying the epithelium. (TB). **Figure 6E:** The apical part of the epithelium and the glands in the lamina propria showing positive reaction by PAS (arrowhead). **Figure 6F:** The apical part of epithelium and underlying glands showing positive reaction by Alcian blue (arrowhead). **Figure 6G:** The wall of the uterus of adult quail consists of long mucosal fold (MF) containing epithelium (Ep) and lamina propria-submucosa (Lp), thick muscular layer (Ms) separated by blood vessels (Bv) and followed by serosa (Se). (Crosmon's Trichrome). **Figure 6H:** Abundant elastic fibers present between the muscle bundles and within the folds (arrowhead). (Wighert's Elastica).

External diameter (μm)	Thickness of wall (μm)	Number of folds / cross section	Height of primary fold (μm)	Width of primary fold (μm)	Height of epithelium (μm)	Thickness of muscular layer (μm)
9148.26 ± 7.8	3686.10 ± 8.9	64.1 ± 2.1	3219.26 ± 6.3	258.82 ± 5.4	23.32 ± 1.91	369.51 ± 3.7

Table 3: The morphometric measurements of the uterus at adult stage.



The sperm host gland

Gross morphology: The sperm host gland was a special area found within the cavity of the uterovaginal junction. It appeared as small recess formed by the cranial part of the vagina (Figure 9A). Its opening was found in the cavity of the uterus, which allowing the stored spermatozoa to ascend toward the infundibulum. The sperms in the sperm host gland were prevented from descending caudally to the lumen of the vagina by the presence of the strong vaginal sphincter. This sphincter was found between the uterus and vagina, which surrounding a narrow lumen (Figure 9B).

Histological analysis: The uterovaginal junction was characterized by abruptly change from the expanded mucosal folds of the uterus into the vaginal mucosa with short folds. This area possessed a special type of vaginal folds. These folds were rich in glands, which are not usually demonstrated in the folds of the vagina (Figure 9C). The sperm host gland was found at the uterovaginal junction separated from the lumen of the vagina by a wedge shaped mucosal ridge and were composed of a single layer of columnar epithelium (Figure 9D).

Scanning electron microscopy: The uterovaginal junction (sperm host gland) showed transition of the mucosal folds from longitudinal folds of the uterus to complex interconnecting folds of the vagina (Figure 10A). The mucous membrane of this area was covered densely with cilia. Careful examination demonstrated many spermatozoa inserted between the ciliated and microvillous cells at sperm host glands (Figure 10B). The sperms arranged solitary on the lining or were gathered into clusters, laid freely in the cavity and had their heads oriented towards the distal portion of the gland. The weak or abnormal sperms cannot ascend within the oviduct due to presence of cilia that hinder them from ascend toward the infundibulum (Figure 10C). Evidence for engulfment of sperm by epithelial cells of the sperm host gland of quail was noticed at this study. These spermatozoa were collected in groups and begin to autolysis and engulfs from the tail region (Figure 10D).

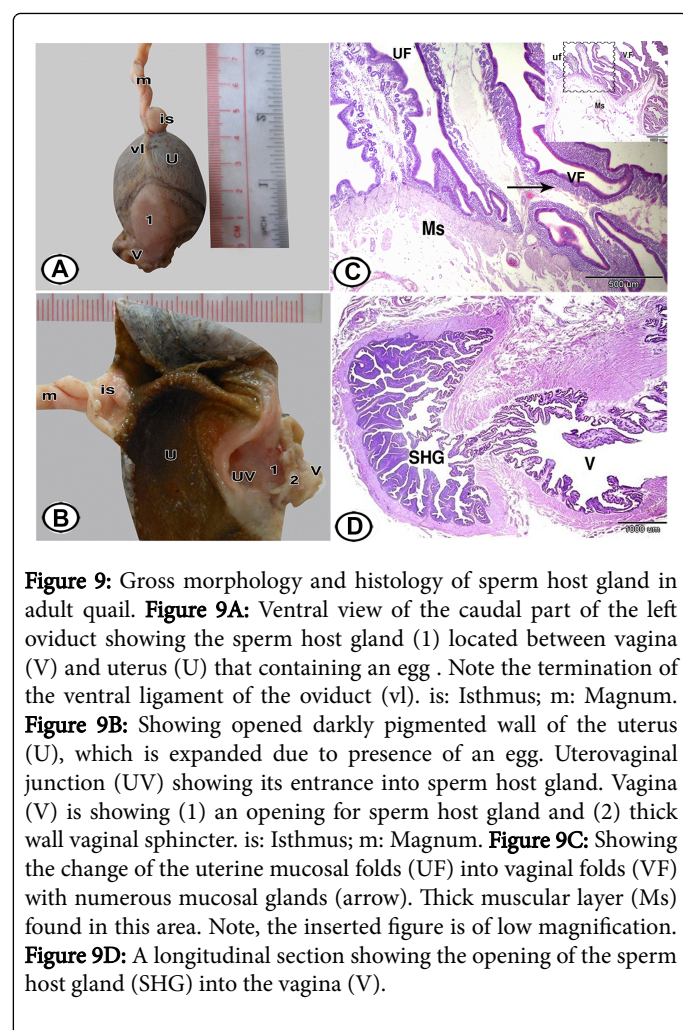


Figure 9: Gross morphology and histology of sperm host gland in adult quail. **Figure 9A:** Ventral view of the caudal part of the left oviduct showing the sperm host gland (1) located between vagina (V) and uterus (U) that containing an egg. Note the termination of the ventral ligament of the oviduct (vl). is: Isthmus; m: Magnum. **Figure 9B:** Showing opened darkly pigmented wall of the uterus (U), which is expanded due to presence of an egg. Uterovaginal junction (UV) showing its entrance into sperm host gland. Vagina (V) is showing (1) an opening for sperm host gland and (2) thick wall vaginal sphincter. is: Isthmus; m: Magnum. **Figure 9C:** Showing the change of the uterine mucosal folds (UF) into vaginal folds (VF) with numerous mucosal glands (arrow). Thick muscular layer (Ms) found in this area. Note, the inserted figure is of low magnification. **Figure 9D:** A longitudinal section showing the opening of the sperm host gland (SHG) into the vagina (V).

Discussion

The present study revealed great histomorphometric, histochemical as well as surface architectural changes of the uterus of Japanese quail during the post-hatching life. In the early stage, the oviduct was classified into cranial, middle and caudal parts. Then, the mucosal folds were significantly increased in number and height associated with increase in height of epithelial cells, which indicated the beginning of rapid growth phase of the oviduct. That is confirmed by the cellular proliferation and intense mitotic activity. The differentiation of the oviduct into distinct parts was completed at 50-days. Similar results are obtained by Lucy and Harshan [17].

The results revealed that the epithelium of the uterus of quail during early undifferentiated stage is consisted of simple columnar cells. Differentiation of ciliated and secretory cells was first appeared in 20-days old. It is well known that the ovarian hormones cause various morphological changes in oviductal epithelial cells. In particular, estrogen induces the primitive stem cells to proliferate into ciliated epithelial cells followed by formation and maturation of tubular glands with their secretory granules [18].

An interesting observation of the present study was the occurrence of secretory granules in the majority of non-ciliated cells. Based on the alcian blue-PAS staining reaction, the epithelium of mature uterus

contained a mixture of acid and neutral mucopolysaccharides. It is known that non-ciliated cells in the uterus of the domestic fowl produce the proteoglycan, ovoglycan, which is a component of the egg shell matrix [19]. In addition, the non-ciliated cells in the domestic fowl also produce osteopontin, a matrix protein involved in the mineralization of the eggshell [20]. The cuticle and shell pigment are thought to be produced by both ciliated and non-ciliated cells [21].

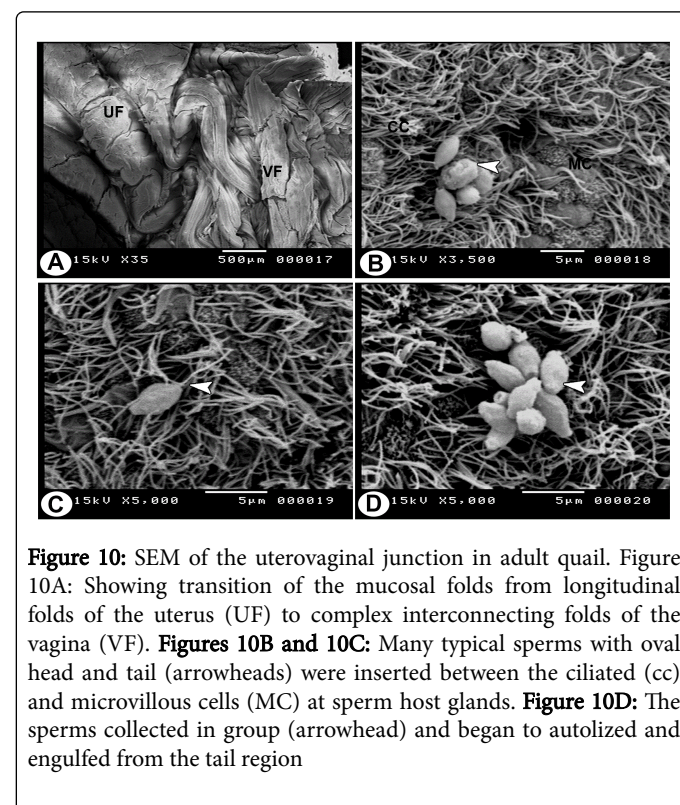


Figure 10: SEM of the uterovaginal junction in adult quail. **Figure 10A:** Showing transition of the mucosal folds from longitudinal folds of the uterus (UF) to complex interconnecting folds of the vagina (VF). **Figures 10B and 10C:** Many typical sperms with oval head and tail (arrowheads) were inserted between the ciliated (cc) and microvillous cells (MC) at sperm host glands. **Figure 10D:** The sperms collected in group (arrowhead) and began to autolyze and engulfed from the tail region

In our study, we suggest that these products are capable of retaining, nutrition and protection of the sperm. The apocrine secretion seem to be the main way, by which the secretion of secretory cells is released and this is identified in scanning electron microscopy by apical protrusions of secretory materials and their construction and detaching from the cell surfaces.

In addition to the surface epithelium, tubular glands located in the lamina propria also make a significant contribution to the secretions of the uterus. In this study, the uterine glands differentiated at 30-days post-hatching and opened directly to the luminal surface by their short ducts. Yamamoto, et al. [22] has shown that gland cells are involved in the transportation of calcium, which is utilized in the calcification of the egg shell.

The overall thickness of the wall of the uterus was increased during the post-hatching period of Japanese quail that may be due to an increase thickening of the tunica muscularis at differentiated and adult stage, and also a greater developed of the mucosal-submucosal folds within this portion [1]. This thick highly vascularized tunica muscularis provide the uterus with the necessary amounts of calcium during egg production. This result coincides with [23] in laying pekin ducks and [24] in hen.

Gilbert [25] found that the capacity for fertilization is retained for about 10 to 14 days after one insemination, so the spermatozoa must

be stored somewhere in the oviduct. Although some sperms are probably stored in the glandular grooves of the neck of the infundibulum [26]. However, the vaginal glands (uterovaginal glands) are generally believed to be the main sites of residence [27]. Sperm host gland of quail is similar in position and function to the previous description of the primary site of sperm storage. It appears as small recess between the uterus and vagina (uterovaginal junction). The sperm host gland stores the sperms that ascend through the oviduct till reach the infundibulum for further fertilization. The sperms in the sperm host gland are prevented from descending caudally to the lumen of the vagina due to the presence of the strong vaginal sphincter.

Sinowatz, et al. [28] demonstrated that the quail sperm storage tubules (SSTs) were positive for steroid dehydrogenases. The presence of other enzymes, particularly those concerned with oxidative pathways, suggests a process of some energy transport in the SSTs. Furthermore, Pal [29] reported intense acid phosphatase activity in the SSTs of the domestic duck, and discussed the role of lipid and this enzyme with respect to the sperm release mechanism. Generally, acid phosphatase is known as a marker enzyme of lysosomes. In any case, further studies are required to elucidate the chemical nature of the substances contained in the SSTs of Japanese quail. We demonstrate sperms eventually engulfed by the cells of sperm host glands in the Japanese quail strongly suggesting a phagocytotic capacity of these cells. Under certain circumstances, it seems to be reasonable that dysfunctioning or relict sperm are appropriately absorbed in the oviductal tract.

Conclusion

The present study provides detailed information on the anatomical, histological, histochemical as well as surface architecture of the uterus of Japanese quail during the post-hatching life, placing emphasis on the secretory epithelium characteristics. The examination of the injected specimens of the serum Indian ink revealed that the uterine glands were richly supplied by arterial blood. Sperm host gland of quail appeared as small recess in the uterovaginal junction that stores the sperms, which ascend through the oviduct till reach the infundibulum for fertilization. Further works on immune histochemical identification of secretory lining epithelium of sperm host gland should be done.

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