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Source: Canadian Journal of Animal Science, 102(3) : 501-508

Published By: Canadian Science Publishing

URL: <https://doi.org/10.1139/cjas-2022-0010>

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# Post-hatch changes in the histological structure of the oviduct and associated 17 $\beta$ -estradiol profiles in guinea hens (*Numida meleagris*)

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## Abstract

There are limited data available on the biology of guinea fowls. The objective of this study was to document post-hatch histological and stereological changes in the oviducts of 56 guinea hens and associated 17 $\beta$ -estradiol profiles until 32 weeks of age and to explore changes in the oviducal mucosal fold as an index of oviducal growth. Mucosal folds in all regions of the oviduct increased significantly ( $P < 0.0001$ ) from 4 to 28 weeks of age. Concentrations of plasma 17 $\beta$ -estradiol increased from 20 to 28 weeks of age, when it stabilized. Mucosal folds in the oviducts of sexually active hens were highest in the uterine region and lowest in the infundibular lip. Intercorrelations among 17 $\beta$ -estradiol concentration, height of mucosal folds, and oviducal weight were strong and positive in these birds. Most of the oviduct epithelium consisted of pseudostratified columnar ciliated epithelium. Both the height of oviducal folds and peripheral estradiol concentrations increased significantly from 20 to 28 weeks of age, which confirms the role of estradiol in oviducal growth in the avian species. The height of oviducal mucosal folds appears to be a good measure of oviducal growth.

**Key words:** histology, guinea fowl, mucosal fold, oviducal growth, oestradiol, stereology

## Résumé

Les données sont limitées sur la biologie des pintades. L'objectif de cette étude était de documenter les changements histologiques et stéréologiques dans les oviductes après l'éclosion de 56 pintades ainsi que les profils de 17 $\beta$ -estradiol associés jusqu'à 32 semaines d'âge, et d'explorer les changements dans les plis des muqueuses de l'oviducte comme indice de la croissance de l'oviducte. Les plis des muqueuses de toutes les régions de l'oviducte ont augmenté de façon significative ( $P < 0,0001$ ) entre les semaines 4 à 28 d'âge. Les concentrations plasmatiques de 17 $\beta$ -estradiol ont augmenté des semaines 20 à 28 d'âge, lorsqu'il s'est stabilisé. Les plis des muqueuses de l'oviducte des poules sexuellement actives étaient les plus nombreux dans la région utérine et les moins nombreux dans la lèvre infundibulaire. Les intercorrélations entre la concentration de 17 $\beta$ -estradiol, la hauteur des plis des muqueuses, et le poids de l'oviducte étaient fortes et positives chez ces oiseaux. La majorité de l'épithélium de l'oviducte consistait d'épithélium cilié disposé en colonnes pseudostratifiées. La hauteur des plis de l'oviducte et les concentrations périphériques d'estradiol ont augmenté de façon significative des semaines 20 à 28 semaines d'âge, ce qui confirme le rôle de l'estradiol dans la croissance de l'oviducte chez les espèces aviaires. La hauteur des plis des muqueuses dans l'oviducte semble être une bonne mesure de la croissance de l'oviducte. [Traduit par la Rédaction]

**Mots-clés :** histologie, pintade, pli de la muqueuse, croissance de l'oviducte, œstradiol/estradiol, stéréologie

## Introduction

The oviduct is a highly convoluted muscular duct that is involved in the transport of the ovum away from the ovary, fertilization of the ovum, and the deposition of albumen, membranes, and shell into the ovum to form the finished egg (Hodges 1974). The oviduct is suspended from the left side of the abdominal cavity by the dorsal ligament, which is a thin, folded, peritoneal membrane attached dorsally to the inner

surface of the abdominal cavity, running caudally from the region of the fourth thoracic rib to the region of the cloaca (Bradley and Charnock 1960) and ventrally to the upper surface of the oviduct.

The normal functional oviduct of the fowl develops from the Mullerian duct on the left-hand side. Right oviducts occur frequently in normal birds as small, sometimes cystic, remnants (Winter 1958), but fully developed right oviducts do

occur (Hodges 1974). The oviduct consists of five distinct regions: the infundibulum, the magnum, the isthmus, the shell gland (or uterus), and the vagina (Hodges 1974). Although the basic structure of each of these regions is the same, the development of different layers varies from region to region (Hodges 1974).

Most estradiol produced by the ovary originates from pre-hierarchical follicles (Johnson 2000). Preovulatory follicle secretion of estradiol, however, increases in each of the four largest follicles 3 to 6 h before ovulation and is greatest in the third and fourth largest follicles (Johnson 2000). An increase in circulating estrogen has been reported to be associated with oviduct development (Renema et al. 1998; Buchanan et al. 2000).

The structure and function of some parts of the oviduct have been described in the following birds: domestic fowl (*Gallus gallus*) (Bakst and Howarth 1975; Bakst 1978); Japanese quail (*Coturnix japonica*) (Eroschenko and Wilson 1974); pied myna (*Gracupica contra*) (Gupta and Maiti 1987); and immature ostrich (*Struthio camelus*) (Madekurozwa 2005). Maturation of the magnum has also been described in domestic fowl (Yu and Marquardt 1973), quail (Pageaux et al. 1986), and ostrich (Madekurozwa 2002).

Information on the histology of the oviduct and associated estradiol changes in the guinea hen is unavailable. In addition, most of the studies conducted on the histological structure of the avian oviduct are descriptive, without any stereological analysis of post-hatch histological changes. The objective of this study was to document these changes and explore changes in the oviducal mucosal fold as an index of oviducal growth. The study also documented associated changes in  $17\beta$ -estradiol profiles until 32 weeks of age, as this hormone has been implicated in oviducal growth in the avian species.

## Materials and methods

### Experimental site, animals, and management

The pearl variety of the local guinea hen (*Numida meleagris*) was used for the study. Birds were brooded for 6 weeks, and brooding management and the lighting program were as previously described in Abdul-Rahman et al. (2017). Feed and water were supplied ad libitum. Day-old keets were fed ground maize in flat feeders, followed by a starter ration (day 2 to 8 weeks of age), grower ration (9–21 weeks of age), and then a layer feed until the end of the experiment. The starter rations (22% crude protein and 3000 kcal ME/kg diet; Nahashon et al. 2007), grower rations (19% crude protein and 3100 kcal ME/kg diet; Nahashon et al. 2007), and layer rations (17.5% crude protein and 2800 kcal ME/kg diet) were obtained from a commercial feed supplier (Agricare Ghana Limited, Kumasi, Ghana).

The birds were raised in Nyankpala, Tamale (Ghana). Nyankpala lies on latitude  $9^{\circ}69'N$  and longitude  $0^{\circ}83'W$ . Temperatures range from 22 to  $35^{\circ}C$  (Abdul-Rahman et al. 2016). The area lies in the Guinea Savannah zone and has almost equal amounts of light and darkness (12L:12D) throughout the year.

### Experimental procedure

A total of 56 guinea hens (7 per age group) were bled at 4, 8, 12, 16, 20, 24, 28, and 32 weeks of age. Two milliliters of blood was collected from the wing vein into EDTA vacutainer tubes and spun at 7100g for 3 min at room temperature ( $18$ – $25^{\circ}C$ ). Plasma was then pipetted into a 1.5 mL microcentrifuge tube and stored at  $-20^{\circ}C$  until subsequently analyzed for  $17\beta$ -estradiol. Prior to bleeding, five birds of each age were weighed and then sacrificed by cervical dislocation after bleeding. The oviducts of these birds were completely freed from the adjoining ligaments and fascia, weighed, and fixed in 10% formalin overnight for histology. Relative oviduct weight was also estimated using the formula proposed by Mishra et al. (2014) ((Oviduct weight/body weight)  $\times$  100). All procedures used were approved by the University for Development Studies Institutional Review Board and followed the Guide for the Care and Use of Laboratory Animals (eighth edition, National Academies Press).

### Histological techniques, general histology of the oviduct, and measurement of height of oviducal folds and concentration of $17\beta$ -estradiol

As the histological techniques used in the present study have been previously described (Abdul-Rahman et al. 2016), only a brief description is given here. Before processing, the oviducts of birds of various ages (4–32 weeks of age) were cut into sections and designated A, B, C, D, and E, which corresponded to the areas that differentiated into infundibulum, magnum, isthmus, vagina, and uterus, respectively, in the sexually mature bird. These sections were fixed in 10% formalin, dehydrated in absolute ethanol, and embedded in paraffin wax. They were then sectioned into  $5\ \mu m$  thickness using a microtome (RM2125 RTS; Leica Biosystems, Wetzlar, Germany), floated onto poly-L-lysine subbed slides (VWR International, Leuven, Belgium), and stained in eosin and Mayer's hematoxylin.

The height of mucosal folds in various sections of the oviduct was measured as the distance from the top of the epithelial cells on the summit of each fold to the area within the mucosa that corresponds to the surface of the epithelial cells in the depressions on either side of the fold using ImageJ software (National Institutes of Health, Bethesda, Maryland, USA) as previously described (Abdul-Rahman et al. 2016). Three parts (initial, middle, and terminal) of each section of the oviduct were used. In each part, 90% (10–15) of the folds present were randomly selected for measurement. In all, 30–45 folds per section of the oviduct per bird were measured and the reading per bird for each section was an average of these 30–45 folds. General histological changes in the oviduct were also described.

Peripheral  $17\beta$ -estradiol concentration was measured by radioimmunoassay using the Oestradiol Maia Kit (RADIM Diagnostics, Colchester, Vermont, USA). This assay was validated for guinea fowl in a previous study (Abdul-Rahman et al. 2016). All samples were evaluated in one assay, and the intra-

assay coefficient of variation was 9.1%. The assay sensitivity was 5.1 pg/mL.

### Statistical analysis

The data were evaluated for normality of variance and homogeneity using Shapiro–Wilk’s *W* and Levene’s tests, respectively. Variances were generally not homogeneous. Age-related changes in ovarian histology and associated 17β-estradiol profiles in guinea hens were therefore analyzed using the Kruskal–Wallis test and medians separated using the Mann–Whitney *U* test. Data were presented as median (interquartile range). Spearman’s rank correlation was also used to determine the relationships among peripheral 17β-estradiol concentration, oviducal weight, and height of oviducal folds. All comparisons were done at 5% level of significance.

### Results

Differentiation of the various parts of the oviduct into the respective regions was generally observed from 20 weeks of age. The sections that differentiated into the respective parts were identified based on their relative location, and the folds were measured to determine age-related changes in the various sections of the duct. All parts were correctly identified, except subsections of the infundibulum. The infundibulum was recorded as the infundibular neck from the onset since sections from only this region were identified and processed. It became possible to partition the infundibulum into lip, neck, and transitional zones from 20 weeks of age.

Developmental changes in the height of mucosal folds in various sections of the oviduct in growing female guinea fowl are presented in **Table 1**. The height of mucosal folds exhibited similar patterns of growth along the entire length of the oviduct. The mucosal folds of the magnum (Kruskal–Wallis test  $\chi^2 = 272.453$ , *df* = 7, *P* < 0.0001), isthmus (Kruskal–Wallis test  $\chi^2 = 280.171$ , *df* = 7, *P* < .0001), and vagina (Kruskal–Wallis test  $\chi^2 = 283.855$ , *df* = 7, *P* < 0.0001) increased significantly in height from 4 to 28 weeks of age. Similar patterns of increases were noticed in the infundibular lip region (Kruskal–Wallis test  $\chi^2 = 81.499$ , *df* = 3, *P* < 0.0001) and neck region (Kruskal–Wallis test  $\chi^2 = 168.184$ , *df* = 6, *P* < 0.0001), except that mucosal folds first emerged at 8 weeks of age in the neck, while in the lip they could only be distinguished from 20 weeks of age.

Uterine folds on the other hand increased in size (Kruskal–Wallis test  $\chi^2 = 283.298$ , *df* = 7, *P* < 0.0001) from 4 to 12 weeks of age, 16 to 20 weeks of age, and 24 to 32 weeks of age. Oviducal weight also increased significantly from 4 weeks of age (Kruskal–Wallis test  $\chi^2 = 275.178$ , *df* = 6, *P* < 0.0001) and stabilized from 28 weeks of age (**Fig. 1**), whereas body weight rose significantly (Kruskal–Wallis test  $\chi^2 = 339.241$ , *df* = 7, *P* < 0.0001) from 4 to 32 weeks of age (**Fig. 2**). Relative oviducal weight, however, increased only minimally from 4 to 24 weeks of age and increased significantly (Kruskal–Wallis test  $\chi^2 = 2.806$ , *df* = 7, *P* < 0.001) at 28 and 32 weeks of age (**Fig. 3**).

The wall of the oviduct was made up of seven layers, from exterior to interior (**Fig. 4A**). These consisted of a thin serous

**Table 1.** Post-hatch changes in the height of mucosal folds in various sections of the oviduct in local guinea hens.

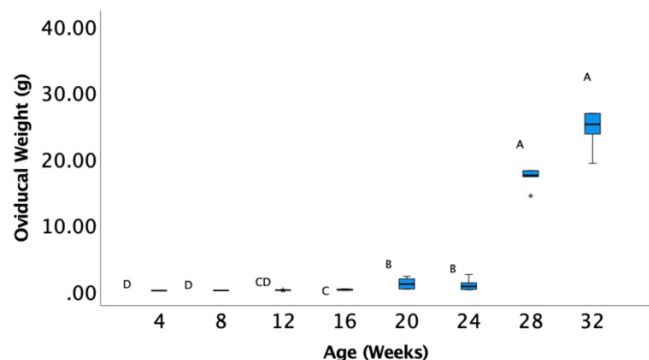
Section of oviduct (median (interquartile range))	Age (weeks)							
	4	8	12	16	20	24	28	32
Infundibular lip (μm)	–	–	–	–	30.1 (27.8–34.7) <sup>f</sup>	51.5 (36.0–59.6) <sup>b</sup>	135.6 (111.3–168.9) <sup>a</sup>	141.6 (130.0–186.1) <sup>a</sup>
Infundibular neck (μm)	–	22.3 (13.8–25.3) <sup>f</sup>	61.5 (47.8–88.8) <sup>e</sup>	125.1 (105.3–155.1) <sup>d</sup>	172.1 (109.4–192.1) <sup>c</sup>	203.7 (186.6–246.9) <sup>b</sup>	330.3 (293.7–417.5) <sup>a</sup>	362.7 (320.8–440) <sup>a</sup>
Magnum (μm)	70.6 (53.2–79.6) <sup>g</sup>	119.9 (98.7–143.1) <sup>f</sup>	185.6 (161.5–227.1) <sup>e</sup>	250.4 (205.7–294.1) <sup>d</sup>	386.0 (299.8–498.6) <sup>c</sup>	562.2 (485.7–901.1) <sup>b</sup>	1930.2 (1464.4–2375.1) <sup>a</sup>	1016.2 (834.9–2496.4) <sup>a</sup>
Isthmus (μm)	33.1 (28.1–37.7) <sup>f</sup>	177.9 (138.8–230.5) <sup>e</sup>	157.2 (130.4–244.6) <sup>e</sup>	237.1 (189.4–284.5) <sup>d</sup>	449.9 (336.6–1072.4) <sup>c</sup>	740.7 (541.8–914.3) <sup>b</sup>	1684.8 (1459.0–2101.8) <sup>a</sup>	1520.3 (1306.4–1787.0) <sup>a</sup>
Uterus (μm)	47.8 (41.9–57.1) <sup>f</sup>	101.5 (85.2–137.90) <sup>e</sup>	205 (164.6–231.6) <sup>d</sup>	200.8 (178.3–223.2) <sup>d</sup>	894.1 (585.3–792.0) <sup>c</sup>	1058.3 (711.4–1626.5) <sup>c</sup>	1956.9 (1078.5–2464.1) <sup>b</sup>	2212.0 (1783.8–3200.9) <sup>a</sup>
Vagina (μm)	35.6 (28.6–41.6) <sup>g</sup>	92.6 (75.7–116.1) <sup>f</sup>	115.6 (92.8–155.6) <sup>e</sup>	175.6 (142.7–206.2) <sup>d</sup>	789.9 (636.3–911.0) <sup>c</sup>	889.2 (799.8–1015.4) <sup>b</sup>	1308.0 (1068.6–1913.8) <sup>a</sup>	1334.8 (1039.2–1677.5) <sup>a</sup>

**Note:** Medians (interquartile range) within a row with different superscripts (a, b, c, d, e, f, g) are significantly different (*P* < 0.05). Median (interquartile range) per parameter per age group involved five birds.

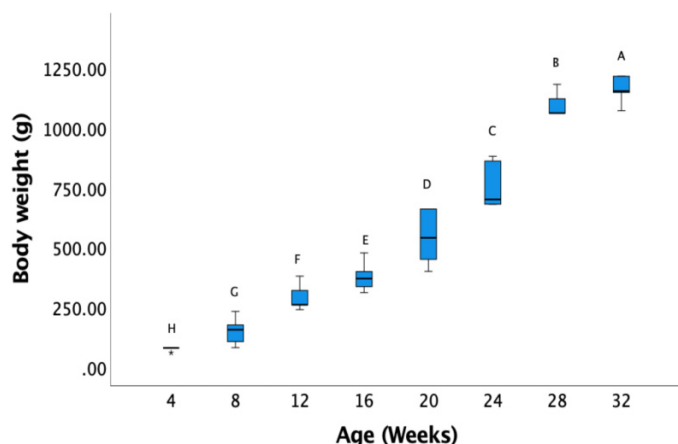
Mucosal folds emerged in the infundibular region from 8 weeks of age but continued to be absent in the infundibular lip until 20 weeks of age when they emerged for the first time in this region.



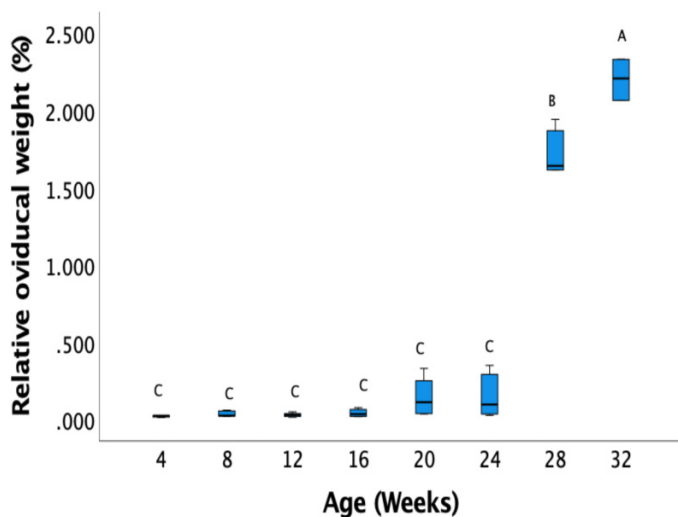
**Fig. 1.** Age-related changes in oviducal weight in local guinea hens. Medians (interquartile range) having no common letter are significantly ( $P < 0.05$ ) different. [Colour online.]



**Fig. 2.** Age-related changes in body weight in local guinea hens. Medians (interquartile range) having no common letter are significantly ( $P < 0.05$ ) different. [Colour online.]



**Fig. 3.** Age-related changes in relative oviducal weight in local guinea hens. Medians (interquartile range) having no common letter are significantly ( $P < 0.05$ ) different. [Colour online.]



epithelium; a layer of longitudinal muscle fiber; a layer of connective tissue containing the larger blood vessels; a layer of circular muscle fibers; a second layer of connective tissue richly supplied with capillaries; a layer of highly glandular mucous membrane in large or small folds, depending on the degree of glandular development; and an internal lining of ciliated epithelium.

Generally, the onset of glandular development was first noticed in the glandular portions of the oviduct from 20 weeks of age (Fig. 4B). From this point on, no major changes were noticed in the histology of the oviduct except the formation, proliferation, and growth in cytoplasmic mass of tubular gland cells and increases in height of mucosal folds in all sections of the duct. These cells attained their maximum size at about 28 weeks of age when birds laid for the first time, which signals sexual maturity. The thickness of the various layers and the nature of glandular epithelium varied in the different sections of the oviduct.

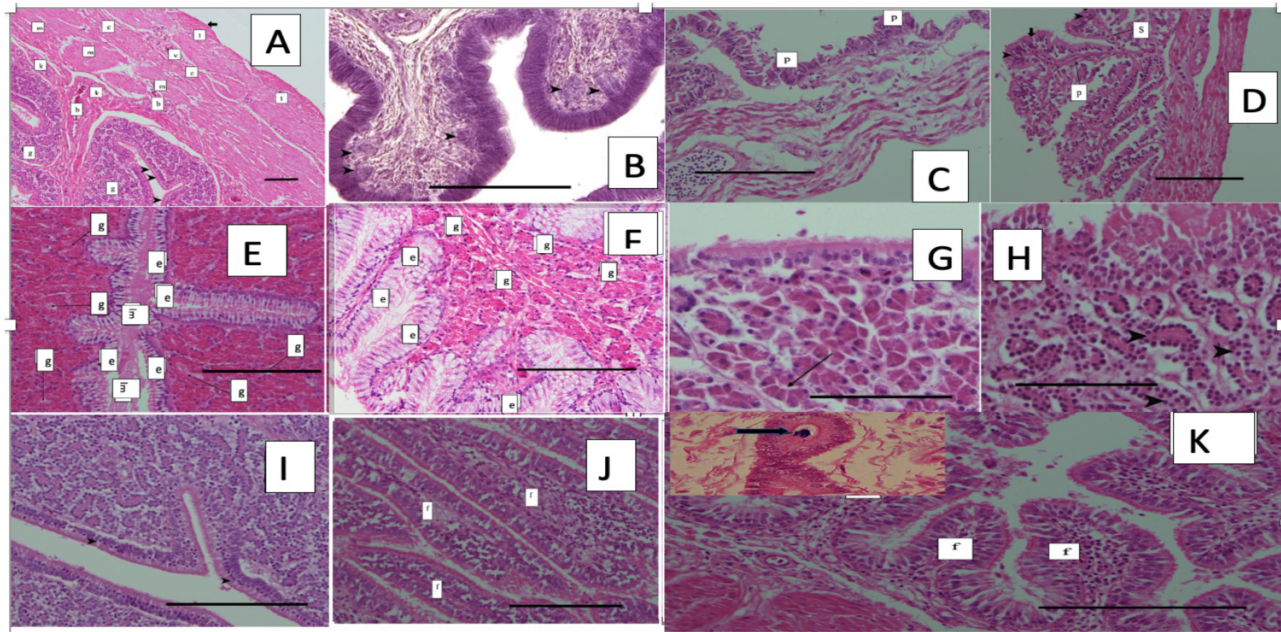
The height of mucosal folds differed significantly in the various regions of the duct in sexually active 32 weeks old birds (Kruskal–Wallis test  $\chi^2 = 513.156$ ,  $df = 9$ ,  $P < 0.0001$ ). In descending order, the mucosal folds were highest in the uterine region (2.3 (1.9–3.0) mm), followed by the magnum (2.0 (1.6–2.3) mm), isthmouterine junction (1.9 (1.5–2.2) mm), isthmus (1.7 (1.3–2.0) mm), and magnum–isthmus junction (1.6 (1.3–2.1) mm), and then the vagina (1.3 (1.0–1.7) mm), uterovaginal junction (0.9 (0.8–1.0) mm), infundibular–magnum junction (0.8 (0.7–1.0) mm), infundibular neck (0.5 (0.4–0.6) mm), and finally, the infundibular lip (0.01 (0.01–0.12) mm).

The general histology of the various sections is described here. The folds in the infundibular lip were very low and only primary folds were identifiable. This region had simple columnar ciliated epithelial cells and no glands were found (Fig. 4C). Both primary and secondary folds were present in the infundibular neck region, where goblet cells appeared to be alternating with ciliated cells and no glands were seen (Fig. 4D). In the transitional region between the infundibulum and the magnum, secretory cells typical of tubular glands (glandular grooves or “gland pouches”) were observed in the shallow depression of the mucosal folds and were shallower than true tubular glands. Except for the infundibular lips and glandular grooves, the rest of the infundibulum was composed of pseudostratified columnar epithelium. The epithelium of the glandular grooves was simple columnar and non-ciliated.

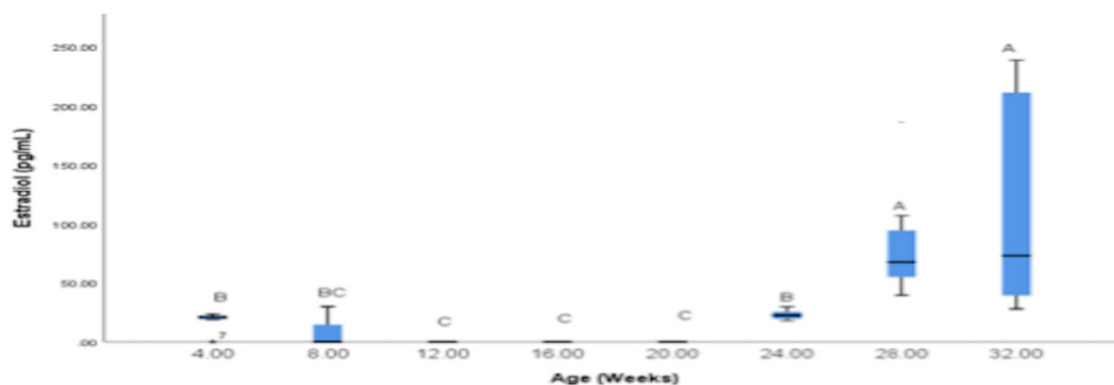
Within the magnum, there was a massive increase in pro- prial glandular tissue leading to more voluminous mucosal folds, which reduced the oviducal lumen to narrow clefts between the folds (Fig. 4E). Only simple folds, with no secondary folds, were found. The excretory gland ducts opened at all points on the surface of these folds. Tubular glands in the magnum were composed of non-goblet non-ciliated cells. The cytoplasm of the cells was filled with fine, dark granules. The goblet cells were as numerous as the ciliated cells in this region.

At the magnum–isthmus junction, pro- prial tubular glands were markedly reduced, and consequently, the mucosal folds were less voluminous and tended to be more complex, with short secondary folds. The epithelium here was taller than those at more anterior levels (Fig. 4F).

**Fig. 4.** Cross-sections of various regions of guinea hen oviduct. (A) Isthmouterine junction showing the general structure of the oviducal wall. From exterior to interior, note the thin serous epithelium (arrowed), longitudinal muscle fiber (l), connective tissue (c) containing the larger blood vessels (v), circular muscle fibers (m), a second layer of connective tissue (k), capillaries (b), a layer of highly glandular mucous membrane in large or small folds (g), and an internal lining of ciliated epithelium (arrowheads). HE  $\times 10$  (scale bar = 100  $\mu\text{m}$ ). (B) Early phase of glandular development (arrowhead) in 20 weeks old oviduct. (C) Infundibular lip showing only low primary (P) folds. (D) Infundibular neck highlighting both primary (P) and secondary (S) folds, and goblet (arrowhead) and ciliated (arrowed) cells. (E) Magnum showing tubular glands (g) and narrow lumen (lm). (F) Magnum  $\times$  isthmus junction showing tubular glands (g) and extensive epithelium (e). (G) Isthmus showing tubular glands (arrowed). (H) Isthmouterine junction showing tubular glands (arrowheads). (I) Uterus showing the uterine epithelium (arrowheads). (J) Vagina highlighting the vaginal folds (f). (K) Uterovaginal junction showing the low mucosal folds (f), HE  $\times 20$  (scale bar = 100  $\mu\text{m}$ ). Insert: sperm storage gland in uterovaginal junction hosting spermatozoa (arrowed), HE  $\times 40$  (scale bar = 25  $\mu\text{m}$ ). [Colour online.]



**Fig. 5.** Changes in peripheral  $17\beta$ -estradiol concentrations from 4 to 32 weeks of age. Medians (interquartile range) having no common letter are significantly ( $P < 0.05$ ) different. [Colour online.]



In the isthmus were tubular glands, unicellular goblet cells, and ciliated cells. There was little histological difference between this region and the albumen-secreting region. Some of the differences noted between the two regions were that the tubular gland in this region did not form as thick a tissue layer as in the magnum region and there appeared to be more cytoplasmic granules in this region than in the mag-

num, even though those in the magnum appeared bigger (Fig. 4G).

The folds between the isthmus and uterus in the isthmouterine junction were shorter (1866.3 (1525.6–2180)  $\mu\text{m}$ ) than those in the uterus (2252.9 (1931–3007.8)  $\mu\text{m}$ ), and the tubular glands were intermediate in character to those in the isthmus and uterus (Fig. 4H). As indicated earlier,

uterine mucosal folds were longer and more complex than the isthmian ones and their proprial gland tissue was less voluminous and narrower. The folds were sometimes bent until they lay parallel to the surface. The pseudostratified columnar ciliated epithelium of the uterus was exceptionally regular, with the nuclei arranged in two rows. The apical cells were ciliated, whereas the basal ones were non-ciliated goblet cells, although both cells extended from the basement membrane to the free border. The tubular glands in this region differed from those in the isthmus in that the cytoplasm was more finely granular and the secretory granules in the cells were finer, more diffused, fewer, and confined to the edges of the cell, particularly toward the lumen (Fig. 4I).

The mucosal folds of the vagina formed longitudinal ridges carrying secondary folds that were particularly regular in arrangement in the lower vaginal region, where they were perpendicular to the parent ridge. The folds were narrower than in most other parts of the oviduct, probably due to the absence of glands, except near the uterovaginal junction, where they were not numerous. The vaginal epithelium was pseudostratified columnar, consisting of ciliated and non-ciliated mucus-secreting goblet cells (Fig. 4J). The goblet cells were sparse in the glandular zone, but formed a much higher proportion than the ciliated cells in other areas. In the uterovaginal junction were glands that were typically short, simple tubules with tall, simple columnar epithelium, although some of these glands tended to show pseudostratification in their epithelium. These glands occurred only in the vicinity of the uterovaginal junction and were less numerous than in other oviducal layers, so they were separated from one another by relatively wide intervals (Fig. 4K). Within the glands, sperm appeared to be clumped together, forming a compact mass (sperm nest; Fig. 4K).

Peripheral  $17\beta$ -estradiol concentration remained stable from 4 to 20 weeks of age (Fig. 5). At 4 weeks of age, however, the levels were higher than those at 12, 16, and 20 weeks of age. Estradiol concentrations registered significant ( $P < 0.05$ ) monthly increases from 20 to 28 weeks of age, when they peaked (57.1 (36.6–103.4) pg/mL) and declined slightly thereafter. Correlations were strong and positive among  $17\beta$ -estradiol concentration, oviducal weight ( $r_s = 0.931$ ,  $P < 0.0001$ ), and height of oviducal mucosal folds ( $r_s = 0.901$ ,  $P < 0.0001$ ). Similarly, oviducal weight was strongly and positively correlated ( $r_s = 0.910$ ,  $P < 0.0001$ ) with the height of oviducal mucosal folds.

## Discussion

The general histology of the oviduct in guinea hens is similar to that reported in the chicken (Winter 1958; Hodges 1974; Surface 2012), turkey (*Meleagris gallopavo*) (Parto et al. 2011), and Japanese quail (Eroschenko and Wilson 1974). The pattern observed in the developing oviduct of the guinea hen is similar to that reported in the chicken by Yu et al. (1972) and Yu and Marquardt (1973) and is essentially a reversal of observations made in regressing quail oviducts (Eroschenko and Wilson 1974).

In a preliminary work, Awotwi (1975) reported that no apparent changes were seen in the histology of guinea fowl

oviducts from 4 weeks of age until sexual maturity at 36 weeks of age. In the present study, however, evidence of glandular development was observed in most birds before sexual maturity (20 weeks of age). The birds used in the present study attained sexual maturity earlier (28 weeks of age) than those used by Awotwi (1975) (36 weeks of age). The earlier attainment of sexual maturity in these birds may be attributed to differences in the way the two flocks of birds were managed. For example, the birds used in the present study were fed starter, grower, and layer rations with crude protein content of 22%, 19%, and 17.5%, respectively, while birds used by Awotwi (1975) were only fed starter rations and layer rations, both with lower content of crude protein (17% and 15%, respectively).

Management factors, including nutrition (Yu et al. 1972; Wilson and Harms 1986) and photoperiod (Morris 1967), could influence the onset of puberty and sexual maturity in birds. Yu and Marquardt (1973) indicated that tubular gland formation began in chickens when the oviduct weighed more than 1 g. This was twice the weight at which glandular formation was initiated in the guinea hen (0.64 g). Oviduct weight was reported to parallel its physiological function (Yu et al. 1971) and could be considered an index of physiological status in the developing oviduct. In guinea hens, the oviduct is therefore likely to function at a much lower weight than in chickens. This is evidenced by the differences in final oviducal weight in laying birds in the two species (51 g vs. 45 g in chickens (Yu and Marquardt 1973) and guinea hens, respectively).

In the quail and chicken, estradiol has been reported to promote the formation of tubular secretory glands and epithelial differentiation (Palmiter and Wrenn 1971; Palmiter 1972; Laugier et al. 1975). Similarly, other studies have reported that administering estrogen stimulates oviduct development, predominantly with the formation and subsequent growth of the tubular gland cells (Oka and Schimke 1969a, 1969b; O'Malley et al. 1969; Yu et al. 1971). Yu and Marquardt (1973) noted that estrogen induces a marked increase in the synthesis of DNA, RNA, and specific egg white proteins. It is therefore not surprising that increasing peripheral estradiol concentrations, noted from 20 weeks of age in the present study, coincided with the start of glandular development at this age. Yu and Marquardt (1973), however, observed that withdrawal of the hormone results in a regression of the oviduct, with involution of tubular glands accompanied by decreases in synthesis of RNA and the specific egg white proteins.

As early as 4 weeks of age, there were folds in all regions of the duct except the anterior portion of the infundibulum. In sexually active guinea fowls, the higher folds found in the uterine and magnum regions may indicate higher secretory activities in these regions of the duct. Palmiter (1972) reported secretion of ovalbumin, conalbumin, ovomucoid, and lysozyme in the oviduct under the influence of estradiol. In contrast, the infundibular lip folds were the lowest in height, contributing nothing except receiving the ovulated egg (Romanoff and Romanoff 1949).

With the exception of the more anterior parts of the oviduct that had simple columnar ciliated cells and were



glandless, all sections of the duct had pseudostratified columnar epithelium consisting of ciliated and non-ciliated goblet cells. The cells forming the tubular glands were found in the transitional zone between the infundibulum and the magnum (indicating the point of emergence of glands in the oviduct), magnum, isthmus, uterus, and finally, in the uterovaginal junction, where a few occurred in the formation of the sperm-storage gland.

The fact that the height of mucosal folds in all parts of the oviduct plateaued from 28 weeks of age, coinciding with the age at which all pullets had laid for the first time, implied that the oviduct continued to grow and develop after hatching until the first egg was laid. The massive increases observed in the height of mucosal folds in all regions of the oviduct from 20 to 28 weeks of age, i.e., the magnum increase by five-fold, the isthmus by four-fold, and the uterus by two-fold, may be linked to the massive increases in secretory activity of these folds, stimulated by the massive increases in estradiol secretion in these birds during this period in preparation for reproductive activities, i.e., from 13.4 pg/mL at 20 weeks of age to a peak of 63.9 pg/mL at 28 weeks of age.

Relative oviduct weight, which indicates the guinea hen's investment in oviducal growth, was relatively stable from 4 weeks of age and only increased significantly during the rapid yolk deposition stages (from 24 to 28 weeks of age) in this species. This indicates that the guinea hen maintains a constant level of investment in stromal, muscular, and epithelial tissues of the oviduct from about 4 weeks of age until puberty, when highly significant investments are made through increased secretion of egg white proteins in preparation for egg laying. The huge increases in the height of oviducal folds at 28 and 32 weeks of age were therefore expected. The increasing body weight associated with increasing estradiol concentrations from 20 weeks of age suggests that ovarian development is advancing in these birds (Renema et al. 1999).

On inspecting the correlational data between plasma estradiol concentration, oviducal weight, and height of oviducal folds, it was found that the coefficient of determination ( $R^2$ ) exceeded 80% in each case. This implies that more than 80% of the variation in oviducal weight and height of oviducal folds may be explained by variation in peripheral estradiol concentrations. Less than 20% of the variation in these variables may therefore be attributed to factors other than peripheral estradiol concentrations.

It has been reported that nutrition influences the growth of the oviduct (Renema et al. 1999). Also, more than 80% of the variation in oviducal weight may be explained by increases in the height of oviducal folds. This phenomenon is interesting as it points to the fact that the bulk of tissue growth in the oviduct during the post-hatch period reflects increases in the height of oviducal folds. Similar to oviduct weight, the height of oviducal folds may therefore be considered a good index of the physiological status of the oviduct.

The general histology of the oviduct in guinea hens is similar to that reported in other birds. Glandular development began at 20 weeks of age, while huge increases in the height of oviducal folds were observed from 20 to 28 weeks of age, the time at which it plateaued. This coincided with the period

when massive increases were recorded in estradiol concentrations, which confirms the role of estradiol in oviducal growth in the avian species. The height of oviducal mucosal folds appears to be a good measure of oviducal growth and may be a good index of the physiological status of the oviduct.

## Acknowledgements

The authors thank Iain Macmillan and Neil Evans of Veterinary Biosciences, University of Glasgow, for their help with histological techniques and radioimmunoassay. The project was partly funded by the Commonwealth Scholarship Commission in the UK and the Association of Commonwealth Universities (CSC ref No. 2009-378).

## Article information

### History dates

Received: 20 January 2022

Accepted: 31 March 2022

Accepted manuscript online: 26 May 2022

Version of record online: 9 August 2022

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