

Light, Scanning and Transmission Electron Microscopical Study on the Oviduct of the Ostrich (*Struthio camelus*)

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Abstract

Nine mature female ostriches (*Struthio camelus*) aged 37- 49 months were used for the present study. The samples were taken during the period from September to November from Al-masreya ostrich farm, immediately after sacrifice and evisceration. Pieces from the different segments of the left oviduct were taken, then fixed in 10% neutral buffered formalin and Bouin's and Susa solutions for the light microscopic study and in 2 % gluteraldehyde for both scanning and transmission E/M examination.

For light microscopic study the slides were stained with Harri's hematoxylen and eosin, Crossman's trichrome stain, Weigert's elastic tissue stain and Periodic acid Schiff technique (PAS). The right oviduct is rudimentary, while the left one is well developed and is formed of five segments, namely: infundibulum, magnum, isthmus, uterus, and vagina.

The scanning E/M revealed that the lining epithelium of the oviduct is folded and formed of pseudostratified columnar ciliated cells. The cilia were slender, dense uniformly arranged, and identical in length.

In magnum, isthmus and uterus the scanning E/M study revealed the presence of openings between the cilia for the secretion of the tubular glands of the lamina propria.

The results obtained were discussed with the available literatures.

Key words

Ostrich, Oviduct, Scanning E/M, Transmission E/M

Introduction

The ostrich, *Struthio camelus*, is the sole species of the family Struthionidae and is the largest living bird. Ostriches provide man with food, clothing, utensils and adornment.

The female ostrich lays an egg every other day. The Red Neck Ostrich can lay 5-15 eggs in the breeding season, while the Blue Neck ostriches lay 30-60 and the Black Neck ones yield 60-120 eggs. Eggs are formed in the left oviduct of the female birds (Bradely, 1928; Cole, 1938; Hodges, 1974; and Bakst, 1978) in fowl; (El-Habbak,1990) in Pekin ducks; (El-Bargeesy,1990) in turkey; (El-Sayed,1994) in geese, duck and pigeon.

In the available literature, only few studies on the morphology and ultrastructure of the ostrich oviduct were found (Muwazi et al.,1982, Hicks-Allderdge, 1998, Madekurozwa, 2005 and Sharaf , 2005). The present work aims to study the histological, histochemical structure as well as the ultrastructure of the oviduct of ostrich. This may be a step for further studies on the oviduct of ostrich.

Material and methods

Nine mature healthy female ostriches (*Struthio camelus*) aged from 37-49 months were used in this study. They were obtained from AL-Masreya ostriches farm on Cairo-Alexandria Desert road.

Samples from the different parts of the left oviduct of each bird were taken directly after scarification and evisceration, then immediately fixed in 10% neutral buffered formalin, Bouin's and Susa fluids for light microscope study and in 2% gluteraldehyde for electron microscope study.

A- For light microscope study

After the routine preparation of the slides, the sections (5-7 micrometer thick) were stained with Harri's hematoxylen and eosin ,Crossman's trichrome, Weigert's elastic tissue, Alcian blue (pH, 2.5) ,Periodic acid Schiff technique (PAS), as described by Drury and Wallington (1980) and Bancroft et al. (1996).

B- For scanning electron microscope

Another tissue samples were taken from different regions of the oviduct and were fixed for 24 hours at room temperature in 2% Gluteraldehyd in 0.1M.Sodium cacodylate (pH 7.4). Then the tissue samples were dehydrated through a graded series of acetone and

then after dried in a critical point dryer. The tissue samples were mounted on aluminum stubs and coated with gold palladium (Reynolds, 1963 and Hayat,1986). The tissue samples were examined with Joel 100CX, scanning electron microscope at 15K.V.

C- For transmission electron microscope

Moreover, some tissue samples were fixed for 24 hours at room temperature in 2% gluteraldehyde, post-fixed in 1% osmium tetroxide and dehydrated in Epon 812. Semi thin sections were cut and stained with toluidine blue for light microscopy. On the lines of Reynolds (1963) and Hayat (1986), ultrathin sections 50-60 nm thick of selected areas were cut, mounted on copper grids and processed for examination using Joel, JEM 100 CXII (TEM).

Results

The right oviduct is rudimentary, while the left one is completely developed. It is divided into five regions namely: infundibulum, magnum, isthmus, uterus, and vagina

1- Infundibulum

The infundibulum is formed of cranial funnel-shaped and caudal tubular parts. The funnel-shaped part is opened toward the left ovary by a wide, slit-like opening, abdominal opening of the oviduct. Its wall is thin and gradually increases in thickness on reaching the tubular part and is folded forming secondary and tertiary folds (Fig 1). The wall of the tubular part is thicker than that of the funnel-shaped part and both parts are lined by pseudostratified ciliated columnar epithelium.

The apical cytoplasm of the lining epithelium of the infundibulum, as well as the connective tissue in the lamina propria and submucosa showed strong PAS-positive reaction. The apical cytoplasm showed also alcianophilic substances.

The scanning electron microscopy (SEM) revealed that the entire surface of the infundibulum is lined by ciliated cells with deep furrows in between. The cilia are cylindrical in shape and identical in length. Clumps of it occur adjacent to the non ciliated areas (Fig 2).

2- The Magnum

The Magnum is the longest portion of the oviduct. Its wall is thick, folded and lined by pseudostratified ciliated columnar epithelium (Fig 3).

The SEM revealed that the mucosa of the magnum is arranged in different folds, separated by deep furrows. The folds were more prominent than that of the infundibulum. The openings of the magnum glands appeared in between the surface epithelium. Most of these openings contain secretory substances of several shapes (Fig 4).

The transmission EM revealed ciliated and non-ciliated cells where the ciliated ones predominate. There were also two cell types lining the luminal surface; light cells and dark cells (Fig 5).

3- Isthmus

The isthmus appears as a short tube, successive to the magnum. Its wall is thicker than that of the magnum. It has also a well developed muscular coat than that of the magnum. The luminal surface is folded and the lamina epithelialis is formed of pseudostratified ciliated columnar epithelium, with some non-ciliated columnar secretory ones.

The apical cytoplasm of the lining epithelium shows strong PAS-positive reaction, and also alcianophilic substances.

The SEM revealed that the mucosa of the isthmus is arranged in a branched convoluted folds separated by deep furrows (Fig 6). The openings of the tubular glands appear in between the surface epithelium and are covered by secretory substances.

4-The uterus

The uterus appears as sac-like dilatation present between the isthmus cranially and the vagina caudally, the wall of the uterus is thicker than that of the isthmus. The luminal surface of the uterus presents tall longitudinal mucosal folds which have lamina epithelialis resembles that of the previous segments. The lining epithelium of the glandular secretory tubules showed negative reaction to alcian blue stain.

The SEM of the uterus revealed that the mucosa of the uterus is arranged in longitudinal folds, which are separated by narrow clefts. The luminal surface is lined by ciliated cells, with several openings of the tubular glands in between (Fig 7). Some of these openings contain secretory substances.

The transmission E/M revealed that the structure of the cilia in the uterus is similar to that of the isthmus. The cytoplasm of the ciliated cells contains mitochondria, rough endoplasmic reticulum, free ribosomes and few electron dense granules. The nuclei were euchromatic with prominent nucleoli (Fig 8).

5-The vagina

It is tube-like structure connected to the uterus cranially and opened on the urodeum of the cloaca caudally. The va-

ginal wall is thicker than that of the other portions of the oviduct.

The luminal surface of the vagina showed thin longitudinally oriented mucosal folds. The lamina propria is formed of fibrous connective tissue with blood vessels and nerves in addition to aggregated lymph nodules in the submucosa (Fig 9).

The transmission EM showed the apical cytoplasm containing secretory granules and both electron dense and electron lucent substances. In addition, it contains rough endoplasmic reticulum, mitochondria, free ribosomes Golgi apparatus. The nuclei were euchromatic with prominent nucleoli (Fig 10, 11)

Discussion

The branched and convoluted folds of the oviductal lumen revealed by SEM results are similar to that mentioned by Fujii (1975) and Bakst (1978) in hen, Sharaf, (2005) in ostrich and Parrizzi et al. (2007) in rhea.

The lamina epithelialis of the oviduct in this study is formed of pseudostratified ciliated columnar cells. In the Bekin duck, El-Habbak (1990) mentioned that the infundibulum epithelium is lined by simple columnar epithelium only.

The strong PAS positive substances in the apical part of the lining epithelium and the lamina propria of the infundibulum, as well as the alciano-philic substances found in the supra-nuclear region of the lining epithelium denotes the secretory activity of this part (agreed with Sharaf, 2005 in ostrich; Aitken and Johnston, 1963; Fujii et al., 1965; and Solomon, 1971 in white Leghorn hens and Fouad, 1970 in Fayomi fowls). Scott and Huang (1941) concluded that

the first albumin layer is formed in the infundibulum neck.

The presence of longitudinal branched folds characterizing the isthmus and vaginal mucosa of this study agreed with the findings of Anita (1971) in Japanese quail and El-Habbak (1990) in Pekin ducks, Bezuidenhout et al. (1995) and Sharaf (2005) in ostrich.

In magnum, isthmus and uterus the SEM revealed the presence of openings among the cilia for the secretion of the tubular glands of the lamina propria.

The present study revealed that the ultrastructure of the surface epithelium showed two types of cells, ciliated and non ciliated granular cells. The ciliated cells are narrow with an expanded apex carrying the cilia. The same results were recorded by Muwazi et al. (1982) in ostrich; Wyburn et al. (1970) in hen and Fertuck and Newstead (1970) in quail. Our results support the conclusion of Muwazi et al., (1982), that the presence of distended tubules of granular endoplasmic reticulum and dense membrane bounded granules in some ciliated cells are taken as evidence of secretory activity.

The propria submucosa in the vagina contains many lymphocytes and lymphoid follicles. In the magnum, isthmus and uterus, the propria possesses simple branched tubular glands like those found in other bird species.

The present investigation showed alcianophilic substances in the cytoplasm of the lining epithelium of the uterus. While the tubular glands in lamina propria showed negative reaction to Alcian blue. The same observations were recorded by Sharaf (2005) in ostrich and El-Habbak (1990) in Pekin ducks. Breen and DeBruyn (1969) sug-

gested that these glands are involved only in the production of the inorganic matrix of the egg.

The neutral mucopolysaccharides, appeared as strong PAS positive substance in the apical cytoplasm of the lining epithelium of the oviduct. On the contrary, the cells lining the tubular glands in lamina propria of the isthmus showed very few faint reactions to PAS y6 (Fujii.,1963 ; and Solomon 1971) in Leghorn hens.

Conclusion

- The right oviduct in ostrich is rudimentary, while the left one is functioning.
- The basic histological and histochemical structure of the five segments of the oviduct in ostrich manifests the secretory activity and follows the same line of other domestic birds with minor species differences.

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Fig (1): Photomicrograph of the infundibulum of ostrich showing: non-branched (arrow heads) and branched (arrows) mucosal folds. P propria submucosa. H&E (X 40)

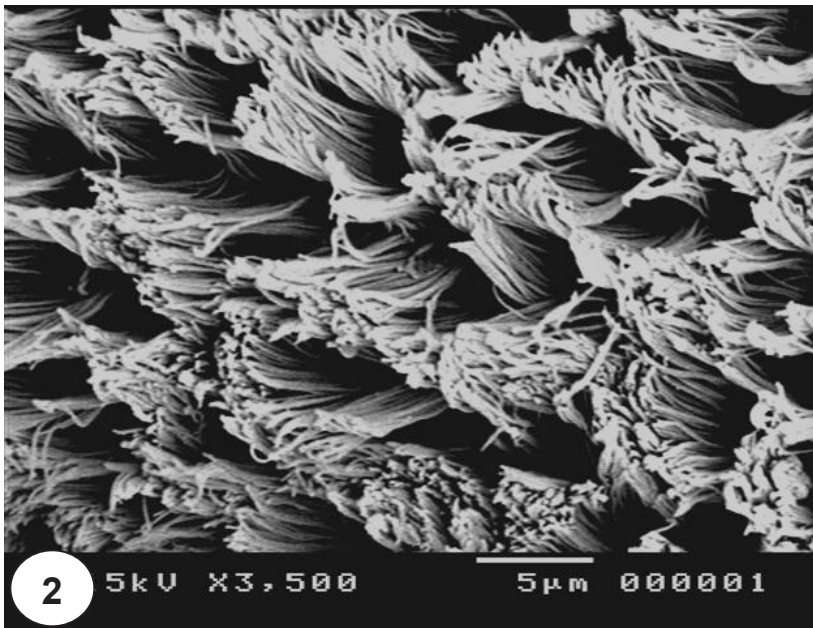


Fig (2): SEM image of the infundibulum of the ostrich showing densely arranged uniform cilia with deep furrows in between (X 3500).

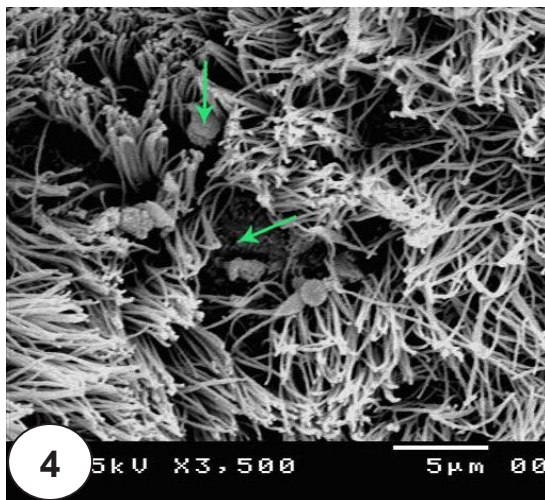
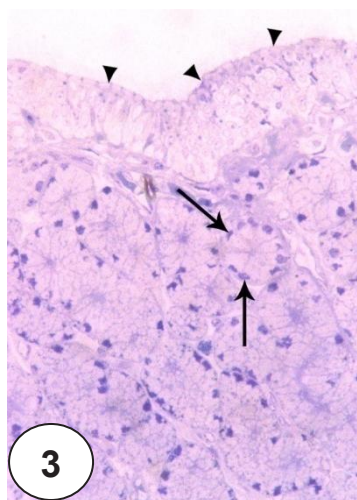


Fig (3): A photomicrograph of the **magnum** of ostrich showing pyramidal cells of tubular glands with acidophilic cytoplasm and flattened basal nuclei (arrows). Notice: ciliated and non-ciliated secretory cells of the lamina epitheliales (arrow heads), Toluidine blue stain. X 1000

Fig (4): SEM image of the **magnum** of ostrich showing tuft of ciliated cells. Cilia appeared identical in form. **Notice:** gland openings (arrows) containing secretory substances. X 3500

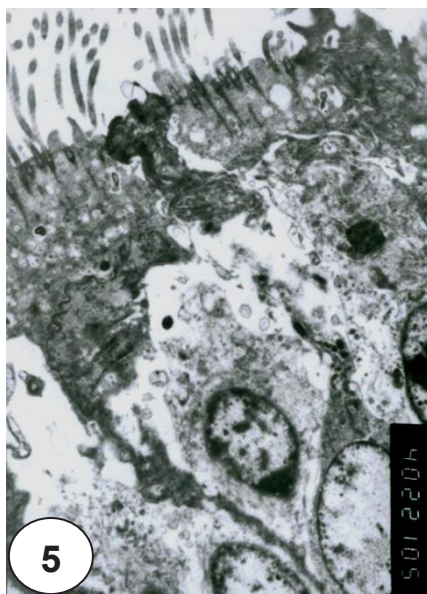


Fig (5): Transmission electron micrograph of lining cells of the **magnum** of mature female ostrich showing dark and light cells. The free surface shows longitudinally sectioned cilia as well as secretory substances (X 4000).

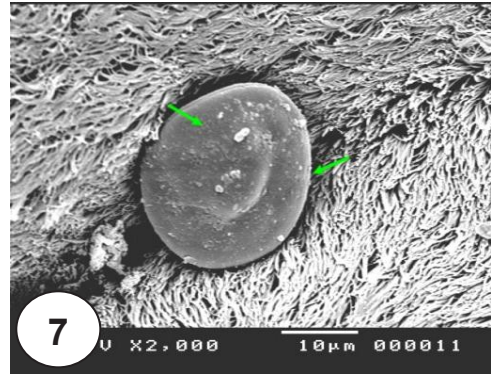
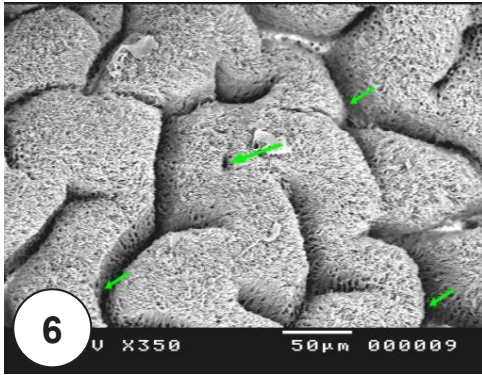


Fig (6): SEM image of the **isthmus** of ostrich showing branching convoluted folds of mucosa, separated by deep furrows (arrows). **Notice:** the openings of the glands (long arrows).

Fig (7): SEM image of the **uterus** of ostrich showing densely arranged cilia of ciliated cells. The mucosal surface is studded by several openings of the glands; some of them contain secretory substance (arrow). X 2000.

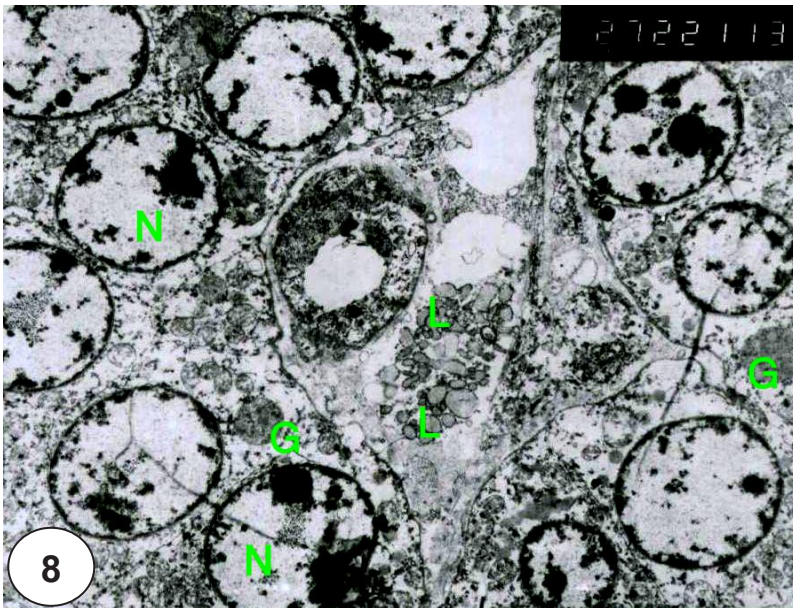


Fig (8): Transmission electron micrograph of the glandular cell in the **uterus** of ostrich showing euchromatic nuclei with prominent nucleoli (N), electron dense granules (G) and lipid droplets (L). X 2700

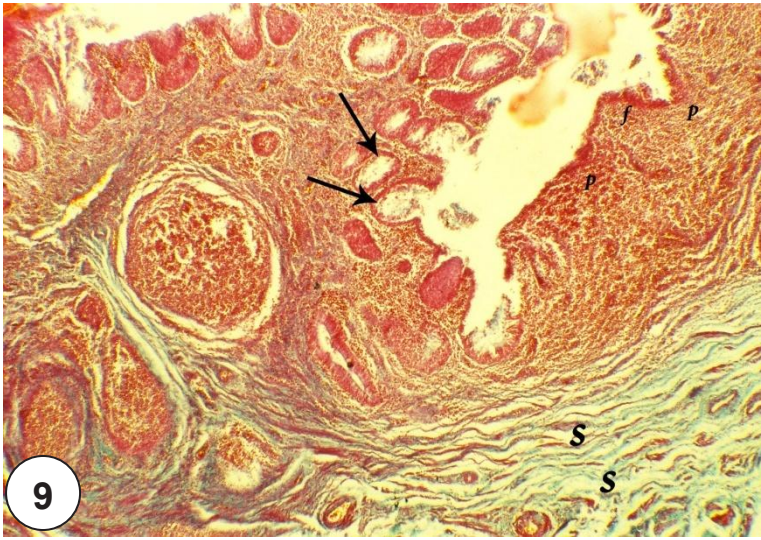


Fig (9): A photomicrograph of the **vagina** of ostrich showing glandular crypts (arrows) in between the longitudinal mucosal folds (f), lamina propria (P), tunica submucosa (S). Notice: aggregated lymph nodules (L).Crossmon's trichrome stain, (X 40).

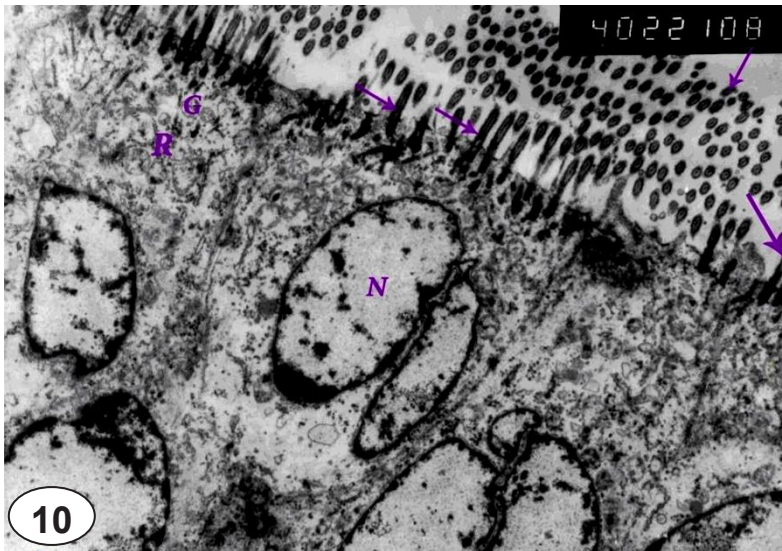


Fig (10): Transmission electron micrograph of ciliated secretory cells lining the **vagina** of ostrich showing longitudinal and cross sectioned cilia (arrows) on the apical surface. Secretory granules in the apical part of the cells (G), euchromatic nuclei (N), rough endoplasmic reticulum (R). X 2700

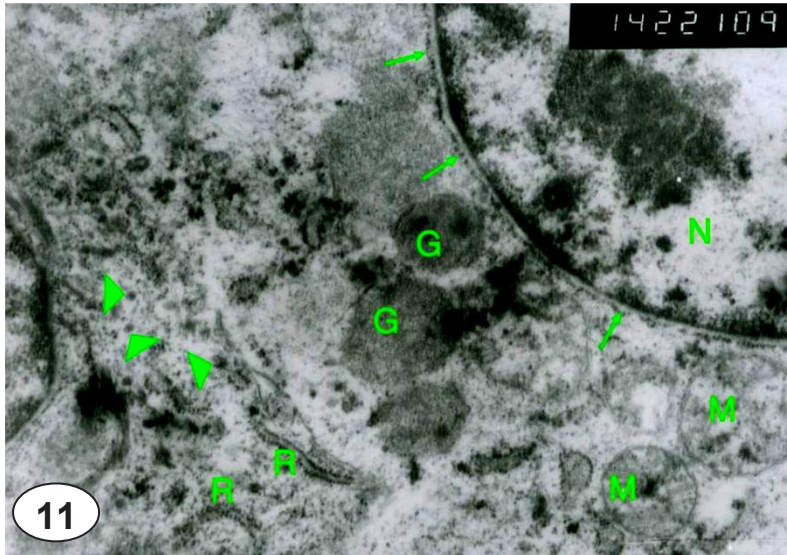


Fig (11): High magnification of a ciliated secretory Cell lining the **vagina** of a mature female ostrich showing: Nucleus (N), containing euchromatin and heterochromatin. Nuclear membrane (arrows), Mitochondria (M), Electron dense granules (G), Rough endoplasmic reticulum (R) , Free ribosomes (arrow heads). X 14000.