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Ultrastructural studies on the isthmus and uterus of oviduct in emu (*Dromaius novaehollandiae*)

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Abstract

The Ultrastructure of isthmus and uterus was studied in eight adult apparently healthy emu birds. The mucosa of isthmus contained large flat and broad mucosal fold. The surface epithelium was pseudostratified columnar ciliated, which contained three types of cells *viz.*, ciliated and non ciliated columnar cells and goblet cells. Clusters of mitochondria were observed around the roots of the cilia. The apical material was bulged into the lumen of the isthmus and gave bleb appearance. The secretory acini were lined with pyramidal cells with spherical nuclei at basal portion of cytoplasm, containing electron dense secretory granules. The mucosa of uterus contained leaf like longitudinal folds, which were compact and tortuous and separated by deep narrow clefts. The mucosal surface was lined by ciliated cells with several openings of the tubular glands opening on to the surface in between and some of these openings contained secretory substances. The surface epithelium was pseudo stratified columnar ciliated epithelium with ciliated, non-ciliated, basal and goblet cells. The ciliated cells presented well developed cilia intermingled with microvilli on their apical surfaces. The tubular glands were lined by pyramidal shaped cells with numerous microvilli on their luminal surface.

Keywords: Isthmus, emu, oviduct, uterus, ultrastructure

Introduction

The emu is the second largest bird and belonged to order Ratite. These birds are reared commercially in many parts of the world for their meat, oil, skin and feathers, which are of high economic value (Sibley and Ahlquist, 1990; Patodkar *et al.*, 2009; Sreedevi *et al.*, 2012; Supriya Shukla *et al.*, 2013) [6, 8, 9]. The ultrastructural studies on the isthmus have been carried out in Ostrich (Sharaf *et al.* 2012) [7]. So the present study was initiated to examine the ultrastructure of the isthmus and uterus in emu (*Dromaius novaehollandiae*).

Materials and Methods

The present work was conducted at the Department of Veterinary Anatomy, College of Veterinary Science, Rajendranagar, Hyderabad. Fresh tissue samples were collected immediately from different regions of the oviduct like isthmus and uterus. For SEM, fixed samples collected from different regions of oviduct were dehydrated in series of graded alcohol and were dried with CPD unit. The dried samples were mounted over the stubs with double-sided conductivity tape and were coated by a thin layer of gold metal over the samples using an automated sputter coater for about 3min (Bozzola *et al.*, 1999) [2]. The samples were scanned under Scanning Electron Microscope (model: JOEL-JSM 5600, JAPAN).

For TEM, the tissues from different regions of the oviduct were dehydrated in series of graded alcohol from 50% to 100% for 40 minutes each, infiltrated in 1:1 alcohol and resin, pure resin and later embedded in pure Spurr resin. Both semi thin and ultra thin sections were cut with a glass knife on a Leica Ultra cut UCT-GA-D/E-1/00 ultra microtome. Semi thin sections of 200-300 nm were stained with Toluidine blue whereas, ultra thin sections (50-70 nm) were mounted on copper grids. Then the sections were stained with saturated aqueous Uranyl acetate for 30 minutes and counter stained with 4% Lead citrate for 20minutes (Bozzola *et al.*, 1999) [2] and were later observed under Transmission Electron Microscope (Model: Hitachi, H-7500, JAPAN). at RUSKA labs, College of veterinary Science, S.V. Veterinary Science, S.V. Veterinary University, Rajendranagar, Hyderabad, India.

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Results and Discussion

Isthmus: Scanning and Transmission Electron microscopy

In SEM, the mucosa of isthmus contained large mucosal folds in longitudinally orientation with deep crypts in between opening to the surface. These large folds were branched and carried secondary and tertiary ones. Similar findings were reported by Parto *et al.* (2011) [5] in turkey. The openings of the tubular glands on the surface epithelium covering the mucosal folds and covered by secretory substances in present study, which agreed with the observations reported by Balash *et al.* (2013) [3] in turkey.

In TEM, the surface epithelium was pseudo stratified ciliated columnar and contained three types of cells *viz.*, ciliated and non ciliated columnar cells and goblet cells. However the surface epithelium was reported to comprise two cell types, ciliated columnar cells and non ciliated secretory cell in turkey by Balash *et al.* (2013) [3]. In present study, the ciliated epithelial cells were less columnar containing microvilli and cilia on their apical surfaces and presented clusters of mitochondria around the roots of the cilia, which agreed nearly the observations of Draper *et al.* (1972) [4] in fowl. However, the microvilli were reported to be presented amongst the cilia as elsewhere in the oviduct epithelium in fowl (Aitken and Johnston, 1963) [1]. In present study, the lining cells were reported to contain nuclei and numerous scattered electron dense granules in their apical expanded parts more towards the apex. Similar observations reported by Draper *et al.* (1972) [4] in fowl. The non ciliated cells associated with GER profiles and cisternae filled with a homogeneous material of medium electron density, which was found to be similar to the observations reported in fowl by Draper *et al.* (1972) [4].

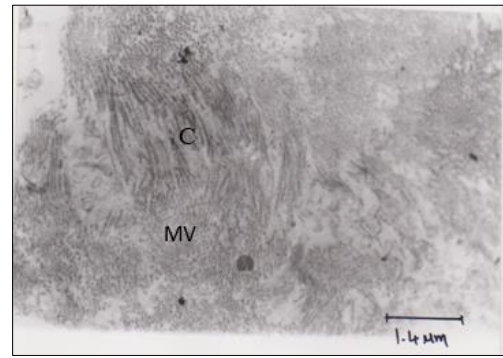


Fig 3: Transmission Electron micrograph (TEM) of the isthmus showing microvilli (MV) and cilia (C) on mucosal surfaces giving bleb appearance in the lumen.X13510

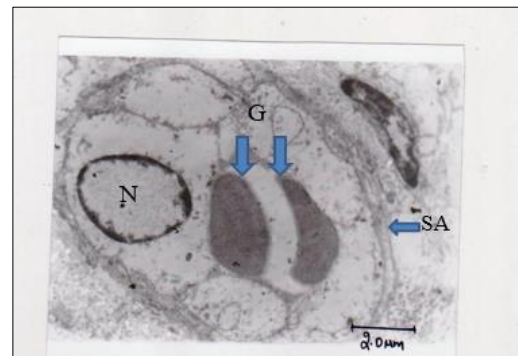


Fig 4: Transmission Electron micrograph (TEM) of the isthmus showing lining epithelial cells of secretory acini (SA) with spherical nucleus (N) and electron dense secretory granules (G) in cytoplasm.X9650

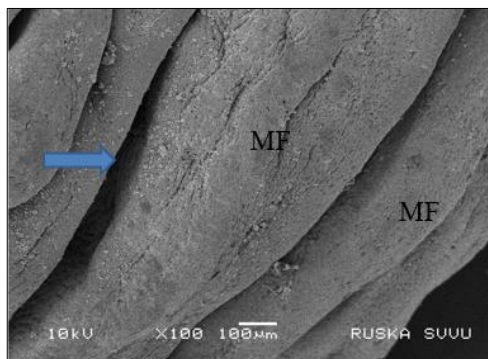


Fig 1: Scanning Electron micrograph (SEM) of the isthmus mucosal surface showing flat and broad mucosal folds (MF) in longitudinally orientation and deep crypts (arrows) in between.X100

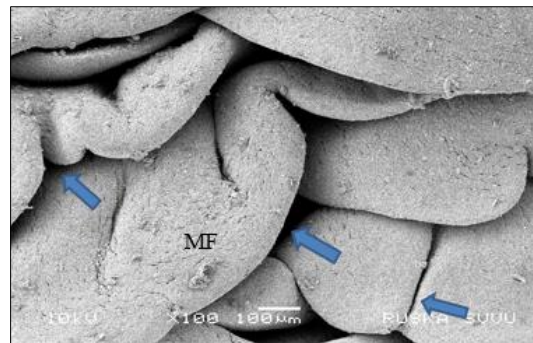


Fig 5: Scanning Electron micrograph (SEM) of the uterus showing compact leaf like tortuous mucosal folds (MF) separated by deep narrow clefts (arrows).X100

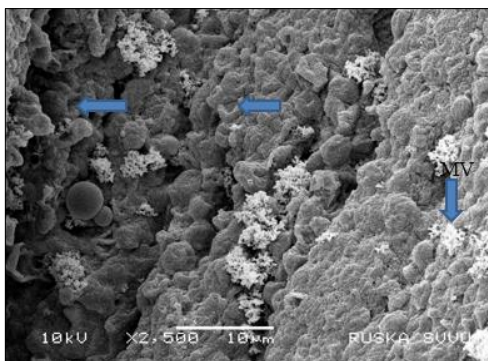


Fig 2: Scanning Electron micrograph (SEM) of the isthmus showing the openings of the tubular glands (arrows) on surface epithelium covering the mucosal folds.X2500, MV-microvilli

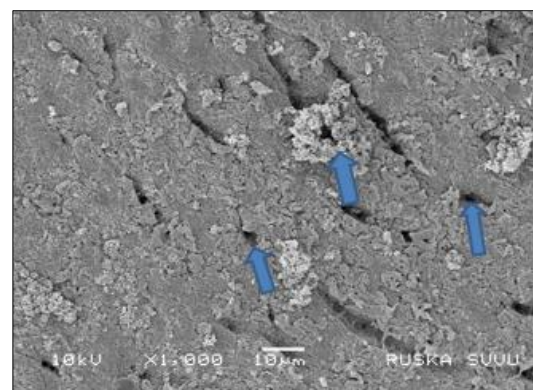


Fig 6: Scanning Electron micrograph (SEM) of the uterus showing several openings of the tubular glands (arrows) on to the mucosal surface.X1000

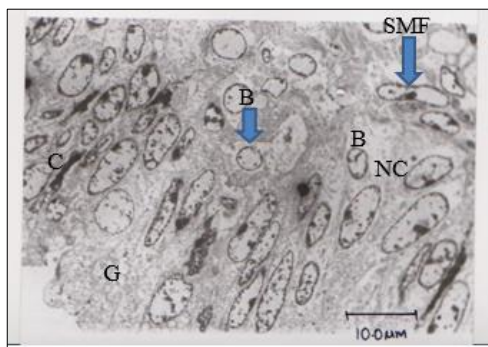


Fig 7: Transmission Electron micrograph (TEM) of the lining epithelium of uterus showing ciliated (C), non-ciliated (NC), basal (B) and goblet (G) cells of elongated euchromatic nuclei (N). Junctional complex (arrows). X1930

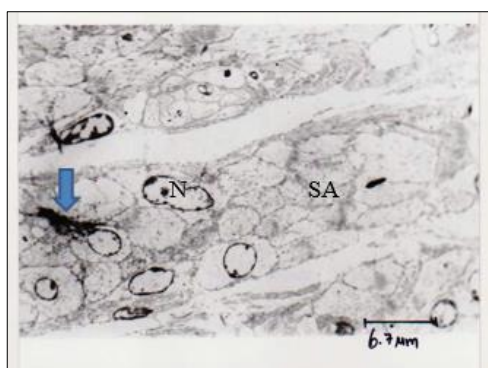


Fig 8: Transmission Electron micrograph (TEM) of the uterus showing electron dense secretory material (arrows) in the apical cytoplasm of the cells and lumen of the secretory acini (SA). X2895
N- nucleus

Uterus

Scanning and Transmission Electron microscopy

In SEM, the mucosa of uterus contained leaf like longitudinal folds, which were compact and tortuous and separated by deep narrow clefts. The mucosa was lined by ciliated cells with several openings of the tubular glands opening on to the surface in between and some of these openings contained secretory substances. Similar findings observed by Saber *et al.* (2009) in ostrich. Our findings also agreed the observations of Parto *et al.* (2011)^[5], who revealed that uterine mucosal folds in turkey were longer and more complex, compressed and longitudinally oriented with voluminous interfold spaces between folds.

In TEM, the surface epithelium was pseudo stratified columnar ciliated epithelium with ciliated, non-ciliated, basal and goblet cells, of which the non-ciliated columnar cells were predominant. While, Johnston *et al.* (1963)^[1], reported that the lining epithelium of uterus in fowl contained ciliated cells, non-ciliated cells and electron lucent cells. According to Jhonson *et al.* (1963)^[1], the ciliated cells exhibit well developed regularly arranged cilia intermingled with microvilli. The ciliated cells contained elongated or irregular shaped nuclei of euchromatic type. In the present study also the ciliated cells exhibit well developed regularly arranged cilia intermingled with microvilli, but the nuclei of ciliated cells positioned apically and that of non-ciliated cells lay basally. The cytoplasm of the ciliated cells contained several mitochondria, rough endoplasmic reticulum, free ribosomes, electron dense granules and bundles of fibrils in the supra nuclear region. Similar observations were reported in fowl by Jhonson *et al.* (1963)^[1] and ostrich by Saber *et al.* (2009).

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