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Microanatomical studies on the infundibulum of oviduct in emu (*Dromaius novaehollandiae*)

Raghu Naik, Santhi Lakshmi, Pramod Kumar and KBP Raghavender

Abstract
In the present study, the infundibulum showed two parts *viz*., funnel and tubal parts. The wall of infundibulum was formed by a tunica mucosa, propria-submucosa, tunica muscularis and tunica serosa from within outwards. The mucosa of infundibular funnel contained smaller and broad unbranched mucosal folds, which increased in number towards the tubal part. The mucosa of infundibular tubal presented well developed tall and short primary folds. The surface epithelium of both infundibular funnel and tubal parts was pseudostratified columnar epithelium with goblet cells. Ciliated and non-ciliated tall columnar epithelial cells were predominant in the surface epithelium of the funnel and tubal parts of the infundibulum.

Keywords: Oviduct, infundibulum, emu, microanatomical

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). The ultrastructural studies on the isthmus have been carried out in Ostrich (Sharaf *et al*. 2012). 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 on "Microanatomical studies on the Oviduct of Emu bird (*Dromaius novaehollandiae*)" was conducted at the Department of Veterinary Anatomy, College of Veterinary Science, Rajendranagar, Hyderabad. Fresh tissue samples were collected immediately from different infundibulum, after sacrifice and evisceration of eight adult apparently healthy emu birds, which were obtained from different slaughter houses and poultry farms located in and around Hyderabad. For Microanatomical studies, fresh tissue pieces were collected from infundibulum of the oviduct and fixed in 10% Neutral Buffered Formalin and Bouin's fluids. Paraffin sections of 5-6µ thickness will be obtained from the tissue pieces and subjected to following routine and special histological staining methods. Standard Haematoxylin and Eosin (H & E) method for the routine histological study (Singh and Sulochana, 1997) [18], Van Gieson's method for collagen fibres (Singh and Sulochana, 1997) [18], Masson's Trichrome method for collagen and muscle fibres (Luna, 1968) [26], Wilder's method for demonstration of reticulum (Singh and Sulochana, 1997) [18], Weigert's method for elastic fibres (Singh and Sulochana, 1997) [18], Crossman's Modification of Mallory's triple Stain for collagen and muscle fibres (Singh and Sulochana, 1997) [18], Verhoeff's method for elastic fibres (Singh and Sulochana, 1997) [18], Toluidine blue method for mast cells (Bancroft and Gamble, 2008) [27].

Results and Discussion
In the present study, the infundibulum showed two parts *viz*., funnel and tubal parts, which coincided with the reports of Parto *et al*. (2011) [28] in turkey and Sharaf *et al.* (2012) in ostrich. The wall of infundibulum in the present work was formed by a tunica mucosa, propria-submucosa, tunica muscularis and tunica serosa from within outwards as reported in domestic birds reported by Naragude *et al.* (1999) in RIR birds, Sharaf *et al.* (2012) in ostrich and Deka *et al*. (2014) [16, 20, 3] in duck. In the present study, the mucosa of infundibular funnel contained smaller and broad unbranched mucosal folds, which increased in number towards the tubal part.

These observations were nearly similar to the reports of Sharaf *et al.* (2012) [20], who reported that the infundibular funnel had short, broad mucosal folds, but highly branched and carried small secondary folds in ostrich. The length and number of mucosal folds was reported to be maximum in the cranial part of infundibulum in Punjab white quail by Bansal *et al.* (2010) [1], which is contrary to the present findings. In the present work, the mucosa of infundibular tubal presented well developed tall and short primary folds. The tall primary folds were branched and carried secondary and tertiary folds. These observations were in agreement. These observations were in agreement with the findings in fowl (Bacha and Bacha, 1990; Naragude *et al.* 1999 and Mehta and Guha, 2012), turkey (Parto *et al*., 2011) [28], hen (Mehta and Guha, 2012), duck (Rao, 1994 and Deka *et al.*, 2014), pigeon (Mohammadpour *et al*., 2008) and ostrich (Sharaf *et al.*, 2012) [7, 16, 15, 15, 17, 3, 14, 20]. The short primary folds numbering about 15-20 were observed in between tall mucosal folds. However the mucosal folds were reported to be smaller in Japanese quail by Ghule *et al*. (2010) and club shaped in hen by Bacha and Bacha (1990) and Mehta and Guha (2012) [8, 7, 15]. The surface epithelium of both infundibular funnel and tubal parts was pseudostratified columnar epithelium with goblet cells. Similar finding was reported in domestic duck by Das and Biswal (1968), in fowl by Banks (1981) and Eurell and Frappier (2006), in ostrich by Muwazi, *et al*. (1982) and Saber *et al*. (2009). However Khadem (2014) [23, 24, 4, 13] stated that the mucosa of infundibulum was lined by ciliated pseudo stratified columnar epithelium and sometimes with ciliated simple columnar epithelium at the funnel region in geese. In contrary to the present findings, simple columnar epithelium predominated with non-ciliated columnar cells was found to be noticed in the funnel part by Hodges (1974), Gilbert (1979), Dellmann (1981) in fowl and Mehta and Guha (2012) [11, 5, 2, 15] in hen. Simple columnar ciliated epithelium with ciliated and non-ciliated cells was observed in Punjab white quails (Bansal *et al.*, 2010) and hen (Naraguda *et al.*, 1999) [1], while similar epithelium with goblet cells was reported in fowl by Bacha and Bacha (1990) and duck by Deka *et al.* (2014) [7, 3]. In the present study, ciliated and non-ciliated tall columnar epithelial cells were predominant in the surface epithelium of the funnel and tubal parts of the infundibulum respectively. Similar findings were also reported in domestic duck by Das and Biswal (1968) [23] and in ostrich by Muwazi *et al*. (1982) and Saber *et al*. (2009) [25]. In the ostrich, Sharaf *et al.* (2012) [13, 20] stated that the mucosa of the infundibular funnel was lined with ciliated simple columnar epithelium, while in the tubal part the epithelium was ciliated pseudostratified columnar type and simple columnar variety in bases of the folds. The tunica mucosa of infundibulum was reported to be lined with simple columnar ciliated epithelium in fowl by Hodges (1974), Bacha and Bacha (2000) and Khoklov (2008) and pigeon by Mohammadpour *et al*. (2008) [35, 11, 7, 14]. Mohammadpour *et al*. (2008) [14] also stated that the lining epithelium of infundibulum was simple cuboidal in upper end and ciliated simple columnar in middle and lower ends of infundibulum in turkey. The present study revealed that the lining epithelium of infundibulum had four types of cells *viz*., ciliated, non-ciliated, basal and goblet cells. Similar finding was reported in fowl by Eurell and Dellmann (2006) and Naragude *et al*. (1999) [4, 16]. The epithelium was reported to be contained non-secreting ciliated cells, non-ciliated goblet cells, Secretory cells especially in the depth of the clefts and tubular gland cells by Parto *et al.* (2011) [28] in turkey. However, three types of cells *viz*., ciliated, goblet and basal cells were reported at the base of the grooves in fowl by Bradley (1960), Dellmann (1993) and Naragude *et al.* (1999) [16, 32]. In the present study, the ciliated columnar cells of funnel part presented dark spherical nuclei in their basal portion, while the nuclei of the goblet cells were spherical and lightly stained. The apical surface of epithelial cells presented a thick zone of basal bodies in the surface epithelium. However the nuclei of epithelial cells were reported to be placed centrally in Japanese quail by Ghule *et al.* (2010) [8].

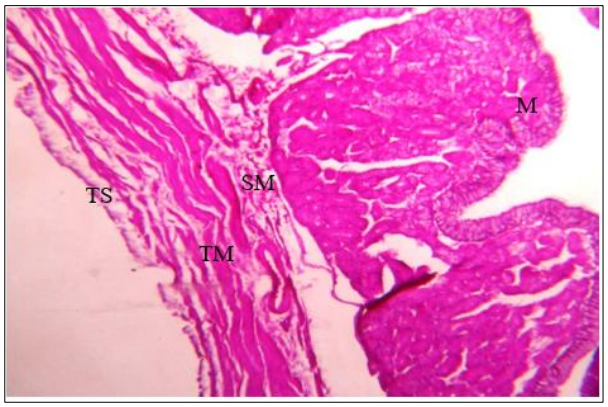

Fig 1: Photomicrograph of the wall of the oviduct showing tunica mucosa (M), submucosa (SM), tunica muscularis (TM), serosa (TS). H&E X 4

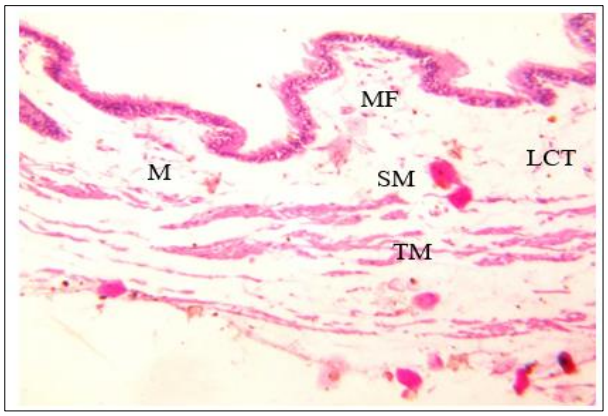

Fig 2: Photomicrograph of wall of infundibular funnel showing unbranched mucosal folds (MF) and showing tunica mucosa (M), propria submucosa (SM), tunica muscularis (TM), and serosa (S). H&E X10 LCT- Loose Connective Tissue.

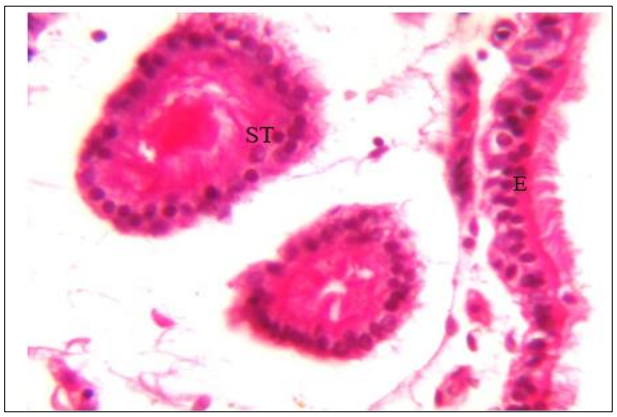

Fig 3: Photomicrograph of infundibular funnel lined with pseudostratified columnar ciliated epithelium (E) predominated with ciliated columnar cells and simple tubular glands (ST) in the underlying connective tissue. H&E X 40

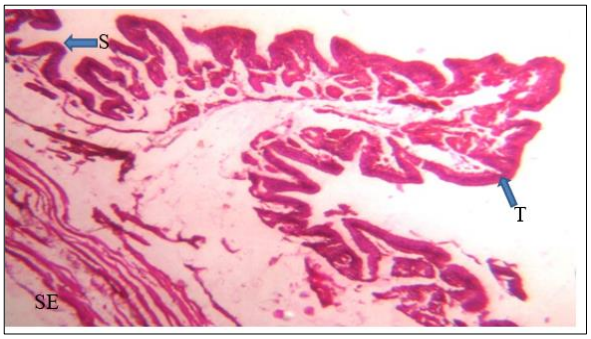

Fig 4: Photomicrograph of infundibular tubal showing tall branched (T) and short unbranched primary folds (S). H&E X4 SE- Serosa

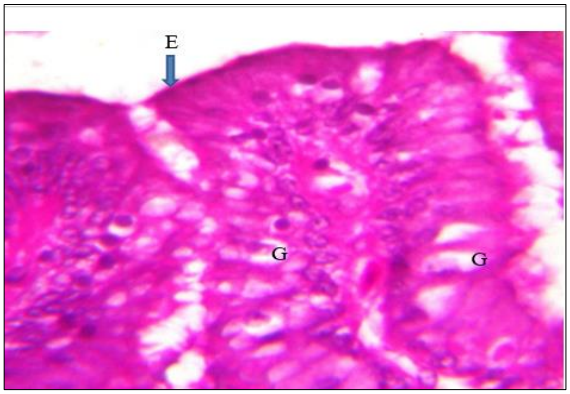

Fig 5a: Photomicrograph of infundibular tubal showing the surface epithelium (E) predominant in non-ciliated columnar epithelial cells. H&E X 40 G-Goblet cells

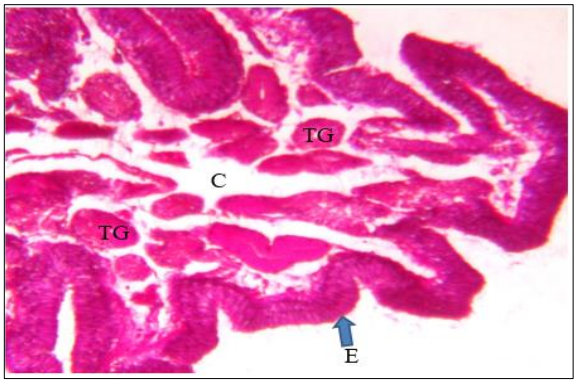

Fig 6: Photomicrograph of infundibular tubal showing simple branched and unbranched tubular glands (TG) beneath the corrugated surface epithelium (E). H&E X10 C- core of the folds

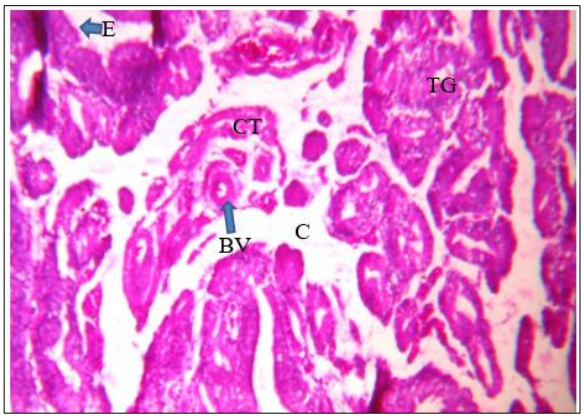

Fig 7: Photomicrograph of the core (C) of the primary folds in infundibular tubal showing vascular connective tissue (CT) and tubular gland units (TG). H&E X40 BV-blood vessels E-epithelium

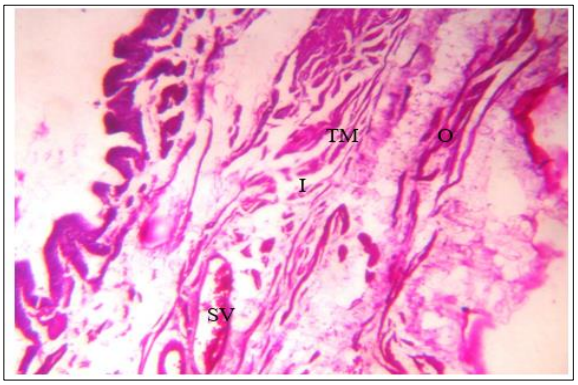

Fig 8: Photomicrograph of infundibular tubal showing well developed tunica muscularis (TM) with inner circular (I) and outer longitudinal (O) layers of smooth muscle fibres. H&E X10 SV- stratum vasculare

The surface epithelium of the infundibular funnel was extended down into the proprial connective tissue and formed simple tubular glands lined with pseudostratified columnar ciliated epithelium. The cells lining the bases of glandular crypts showed long prominent cilia. The glandular units showed acidophilic secretory material in the center of their lumen. The epithelial cells lining the glands showed eosiniphilic cytoplasm and spherical nuclei in central to basal portions. In contrary to present findings, the presence of glands was not reported in laminapropria of infundibular funnel by Das and Biswal (1968) [23] in domestic duck, Hodges (1974) [11] in domestic fowl, in quail by Sayed (2008) and in turkey by Mohammadpour *et al*. (2008). In the present study a rich capillary network and some smooth muscle fibres were observed beneath the basement membrane of the surface epithelium. The surface epithelium the infundibular tubal was extended down into the underlying proprial connective tissue as simple branched or unbranched tubular glands. Similar observations were reported in Japanese quail by Lucy and Harshan (1999), in turkey by Mohammadpour *et al*. (2008) and in duck by Deka *et al*. (2014) [12, 14, 3]. The bases of the glands were lined with tall columnar or pyramidal cells, of which most them were non ciliated type, which is in partial agreement with the findings of Sayed (2000) [19] in quail. The cells showed vacuolated cytoplasm and round vesicular nuclei towards the base with prominent nucleolus. The surface epithelium showed several tubular invaginations (proprial glands) on the tall primary folds, the bases of which presented the openings of the glandular ducts. Similar observations, but the presence of proprial glands only in tubal part was reported by Bansal *et al.* (2010) [1] in Punjab white quail. The thickness of glandular portion was more in tubal part when compared to the funnel part, as reported in mature birds of RIR by Naragude *et al.* (1999) [16]. The core of the tall primary folds contained tubular secretory acini surrounded by proprial connective tissue below the lining epithelium. Similar observations were reported by Mohammadpour *et al*. (2008) [14] in turkey. The centre of the core in both tall and short primary folds contained highly vascular loose connective tissue with few smooth muscle fibres. Lymphocytes and plasma cells were observed in scattered form in the proprial connective tissue in the tubal part of infundibulum, which is in partial agreement with the findings of Mohammadapour and Keshtmandi (2008) in turkey. However, Bradley (1960) and Kimijima (1989) [32, 14] mentioned occasional presence of islands of lymphoid tissue only near the middle of infundibulum in fowl.

The submucosa was reported to be not distinct in the infundibulum of domestic duck (Das and Biswal, 1968) [23] and in ostrich (Muwazi *et al*., 1982) [13]. In the present study also the subepithelial connective tissue was not differentiated into lamina propria and submucosa. The propria-submucosa of infundibular funnel part was composed of loose connective tissue with fine collagen and elastic fibers along with leucocytes, plasma cells and some blood vessels, which concurs with the observations in Japanese quail (Lucy and Harshan, 1999 and Ghule *et al*., 2010) and duck (Deka *et al*., 2014) and and Japanese quail (Ghule *et al*., 2010) [12, 8, 3, 8]. The loose connective tissue stroma of tubal part was highly vascular when compared to funnel part and filled with several simple branched or unbranched tubular glands in subepithelial layer. These findings were coincided with the reports of Lucy and Harshan (1999) and Sayed (2000) in quail and Deka *et al*. (2014) in duck [12, 19, 3].

The tunica muscularis of the infundibulum contained inner circular and outer longitudinal layers of smooth muscle fibres as reported in fowl (King and Mc Lelland., 1975 and Bacha and Bacha., 2000), turkey (Mohammadpour *et al*., 2008 and Parto *et al*., 2011) [35, 28], guinea fowl (Kanchana *et al*., 2010) and duck (Deka *et al*., 2014) [9, 14, 3], The muscle layers of the tubal part were supported by large amount of loose connective tissue and separated by a thin stratum vasculare as reported in fowl by Garg (2006) [6]. The presence of only scattered bundles of outer longitudinal smooth muscles was reported in fowl by Eurell and Frappier (2006) [4]. The tunica muscularis was reported to be less differentiated and represented by strands of smooth muscle fibers in quail by Sayed (2000) [19]. The lymphocytes were scattered in the connective tissue surrounding the blood vessels and among the bundles of smooth muscle in the tunica muscularis. Similar observation was noticed in japanese quail by Lucy and Harshan (2011) [37]. In duck, Lucy (2011) also noticed the presence of lymphoid tissue in the connective tissue among bundles of smooth muscle in the neck region of the infundibulum but in aggregated form.

The serosa was well developed and it was formed by a layer of loose connective tissue with fine collagen, elastic and reticular fibres and covered by mesothelium. Similar observations were reported in fowl (Dellmann, 2006) [4], Punjab white quails (Bansal *et al.*, 2010) and duck (Deka *et al*., 2014) [1, 3].

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