



ISSN (E): 2277- 7695  
ISSN (P): 2349-8242  
NAAS Rating: 5.03  
TPI 2018; 7(1): 576-579  
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www.thepharmajournal.com  
Received: 27-11-2017  
Accepted: 28-12-2017

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## Histochemical studies on the oviduct of emu bird (*Dromaius novaehollandiae*)

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### Abstract

Strong reactivity for PAS was observed in the apical portion and ciliary zone of surface epithelial cells and the cells lining the tubular glands of the mucosal folds of infundibulum, magnum, isthmus, uterus, while in vagina it was limited to basal portion of the lining epithelium. The connective tissue around the glandular units of all the regions of the oviduct except vagina showed strong positive reaction for PAS. The alkaline phosphatase activity was strong in the basement membrane and apical part of lining epithelium of all the regions of the oviduct, submucosal connective tissue and tunica muscularis region of infundibulum, uterus, vagina. Strong reaction for ALP was observed in lamina propria and muscular layer of isthmus, while moderate reaction was noticed in submucosa and muscularis region of magnum. The oil red 'O' staining activity was strong in lining epithelium of the mucosal folds in infundibulum and vagina and mild to moderate in the lining epithelium, lamina propria and submucosal regions of isthmus and glandular units of uterus.

**Keywords:** Histochemical studies, oviduct, emu bird (*Dromaius novaehollandiae*)

### Introduction

The Emu bird is the second largest living bird and is reared commercially in many parts of the world for meat, oil, skin and feathers, which are of high economic value. The oviduct of emu is a relatively a straight tube that extends from the level of the cranial extent of the left ilium to the caudal border of the left pubic bone and it was divided grossly into five regions i.e., infundibulum, magnum, isthmus, uterus and vagina using variations in the mucosal fold pattern in avians (King and Mc Lelland., 1975) <sup>[7]</sup> and emu. The morphology of the avian oviduct have been studied for many years, especially in domestic fowl. However the literature available was focussed mainly on the gross anatomical features of the oviduct in emu bird. Very little information is available on the histochemistry of oviduct and it was restricted to ostrich bird. Hence the present work has been undertaken in order to improve the their production and reproductive efficiency.

### Materials and Methods

The present work was conducted at the Department of Veterinary Anatomy, College of Veterinary Science, Rajendranagar, Hyderabad. The tissue samples were obtained from ten adult apparently healthy Emu birds from slaughter house in Vijayawada and poultry farm in the Hyderabad. Fresh tissue pieces were collected from different regions of the oviduct were obtained immediately after the slaughter. The tissue pieces were fixed in 10% neutral buffered formalin and processed for paraffin sections as per the methods described by Singh and Sulochana (1997) <sup>[13]</sup> for the paraffin sections. For histochemistry the tissue pieces were fixed at 4°C in chilled 10% neutral buffered formalin and frozen sections of 15-20 µm thickness were cut by using a cryostat and stained as per the methods described by Luna (1968) <sup>[8]</sup> i.e., Periodic Acid Schiff (PAS) technique for mucopolysaccharides, Combined Alcian blue - PAS (AB-PAS) technique for Acid and Neutral mucopolysaccharides, Oil Red 'O' in propylene glycol for Lipids and Gomoris' calcium method for Alkaline phosphatase activity and Gomoris' lead method for Acid phosphatase activity (Bancroft and Gamble, 2008) <sup>[5]</sup>.

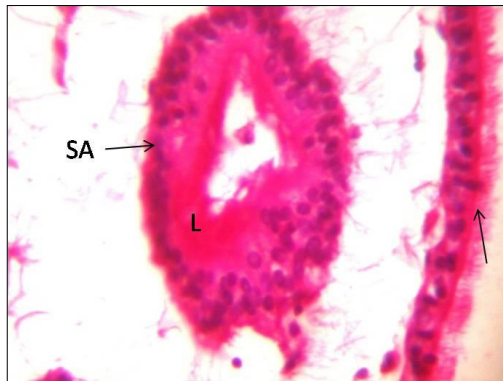
### Results and Discussion

#### 1. Infundibulum

The infundibulum showed positive reaction for PAS and PAS - Alcian blue in the apical portion of the lining epithelium of the infundibulum (Fig.1), as reported by Saber *et al.* (2009) <sup>[11]</sup> in ostrich. In contrary to this Sharaf *et al.* (2012) <sup>[12]</sup> reported a negative PAS reaction in the

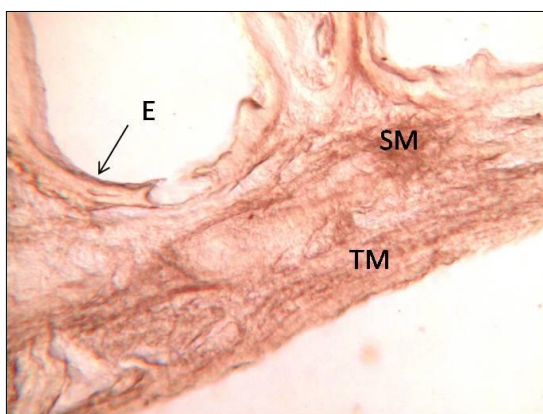
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ciliary zone of surface epithelium in ostrich. The connective tissue of propria-submucosa showed strong PAS positive reaction (Fig. 2), as noticed in ostrich by Sharaf *et al.* (2012) [12]. The ciliary zone in surface epithelium of the infundibular funnel showed a strong PAS positive reaction (Fig.1). The supranuclear regions of the surface epithelial cells in the infundibular tubal and the cells lining the glandular tubules in infundibular funnel and tubal parts exhibited a strong PAS positive reaction. PAS positive reaction was also observed strongly in the luminal borders and the secretions in the lumen of glandular secretory units (Fig.1), which agreed with the findings of Sharaf *et al.* (2012) [12] in ostrich. The secretory material in the lumen of glandular units showed positive reaction for calcium.



**Fig1:** Photomicrograph showing strong PAS positive reaction in apical portion and ciliary zone of the lining epithelium (arrow), luminal border (L) of secretory unit (SA) of the infundibular funnel. PAS X 40

The alkaline phosphatase activity was strong in the apical surface of epithelial cells of the surface epithelium, submucosal connective tissue and tunica muscularis region (Fig. 2) and there was no evidence of acid phosphatase activity in the infundibulum. The oil red ‘O’ staining activity for fat was strong in epithelium and mild to moderate in muscular region.



**Fig 2:** Photomicrograph of the infundibular tubal showing strong alkaline phosphatase (ALP) (arrow) activity in the surface epithelium (E), submucosal connective tissue (SM) and tunica muscularis region (TM). ALP X 10

**2. Magnum**

Intense PAS positive reaction was observed in the lining epithelium and tubular glands due to the presence of glycogen in the cytoplasm of the epithelial cells. Similar findings were reported in fowl by Bharti *et al.* (2012) [6]. However, the

lining epithelium was reported to had only acidic mucopolysaccharides in hen (Davidson *et al.*, 1986), duck (Ozen *et al.*, 2009) [9] and Punjab white quails (Bansal *et al.*, 2010) [4]. A moderate PAS positive reaction was observed in the central part of the mucosal folds. The surface epithelium covering the mucosal folds and glandular crypts contained neutral mucopolysacchrides and exhibited strong PAS and PAS –AB reaction in the supranuclear regions (Fig.3). The apical regions of the of both ciliated and non ciliated epithelial cells and goblet cells lining the magnum showed strong PAS positive stained matter (Fig. 3) as reported by Sharaf *et al.* (2012) [12] in ostrich. In contrary to this, very weak activity for neutral mucopolysaccharides was reported to be observed in the lining epithelium of quail by Bansal *et al.* (2010) [4]. However the ciliated non secretory cells were PAS negative. Most of the glandular epithelium showed strong PAS-positive reaction and the maganal glands were PAS- AB positive (Fig. 3), which agreed with the findings of Aitken (1971) [1] in avians. However the maganal glands were reported to be alcian blue negative in ostrich by Sharaf *et al.* (2012) [12] The connective tissue surrounding the blood vessels exhibited week PAS positive reaction.



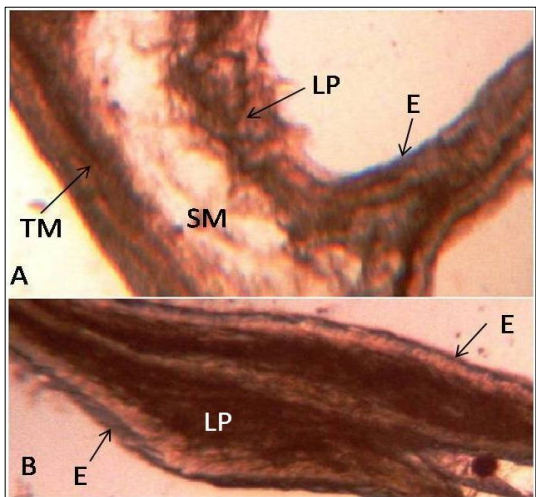
**Fig 3:** Photomicrograph of the magnum showing strong PAS (arrow) reaction in apical portion of surface epithelium (E) of the mucosal folds and proprial glands (TG). PAS-AB X 10

The alkaline phosphatase was strong in the basement membrane and apical part of lining epithelium and moderate in submucosa and muscularis region and there was no acid phosphatase activity. The oil red ‘O’ staining activity for fat was strong in lining epithelium and mild to moderate in propria submucosa and muscularis region as reported by Bansal *et al.* (2010) [4] in quail.

**3. Isthmus**

A positive reaction was observed for the PAS in the mucosal lining of isthmus. The apical portion of surface epithelium of the mucosal folds and proprial glands and their ducts showed strong reaction for PAS as noticed in ostrich by Saber *et al.* (2009) [11]. The staining affinity was more in the deep seated glands than the sub epithelial glands. The connective tissue around the blood vessels and supporting the glandular units presented strong positive reaction for PAS and PAS-AB.

In present study, the apical portion of epithelium lining the proprial glands and glandular ducts showed the presence of both acid and neutral mucopolysaccharides. However, the entire lining epithelium of isthmus was reported to present both acidic and neutral mucopolysaccharides in the proprial glands in hen (Artan and Daghoglu, 1984) [2], duck (Ozen *et al.*, 2009) [9] and Punjab white quail (Bansal *et al.*, 2010) [4].

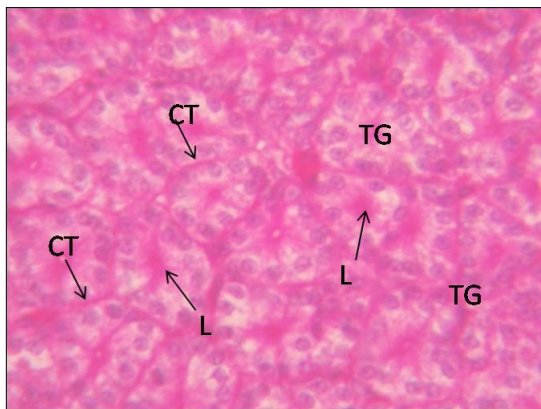


**Fig 4:** Photomicrograph of the wall of isthmus (A) and mucosal fold (B) showing strong alkaline phosphatase (ALP) (arrow) activity in the apical part of lining epithelium (E), lamina propria (LP) and tunica muscular region (TM), mild reaction in submucosa (SM). A&B - ALP X 4 B

The Alkaline phosphatase activity was strong in the apical part of lining epithelium, lamina propria and muscular layer (Fig.4). However mild reaction for ALP was observed in submucosal layer (Fig.4). The acid phosphatase activity was found to be nil in the isthmus. The Oil red 'O' staining activity for fat was mild to moderate in the lining epithelium, lamina propria and propria submucosa regions.

**4. Uterus**

The lining epithelium of the uterine mucosal folds and crypts showed strong PAS positive reaction and PAS - AB positive reactions. However, a weak to moderate reaction for neutral and acid mucopolysaccharides was reported to be observed in the lining epithelium of the glands by Saber *et al.* (2009) [11] in ostrich. The connective tissue around the glandular units and the luminal borders of the secretory cells exhibited strong PAS positive reaction (Fig. 5). The lining epithelium of the glands showed a weak to moderate reaction for neutral and acid mucopolysaccharides. The connective tissue around the glandular secretory tubules and ducts showed positive reaction to PAS - AB stain. The lipid material was abundant in the gland cells and these cells showed strong ACP activity but no ALP activity.

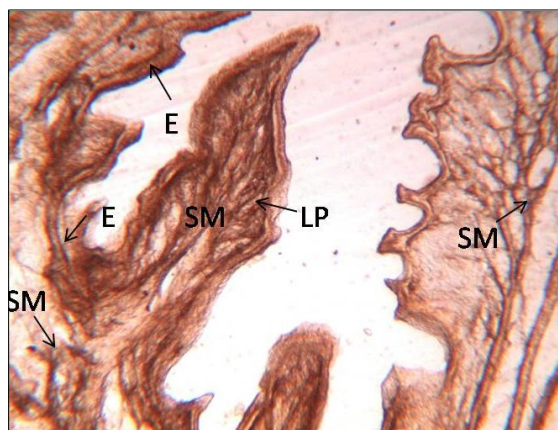


**Fig 5:** Photomicrograph of the uterus showing strong PAS (arrow) positive reaction in the connective tissue (CT) around the glandular units (TG). L-Lumen of secretory units. PAS X 40

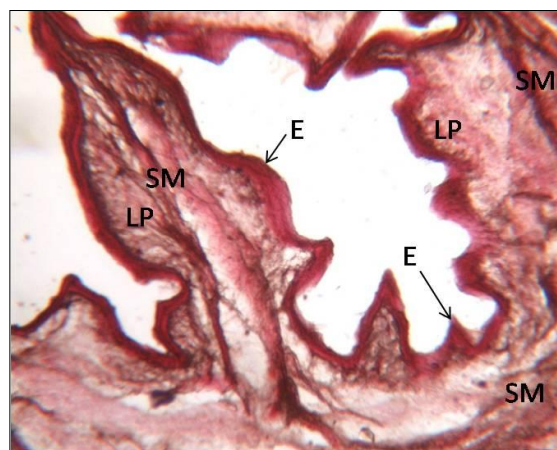
The alkaline phosphatase activity was strong in the apical part of lining epithelium, submucosal region and muscular region and there was no acid phosphatase activity. However, the gland cells reported to contain strong ACP activity but no ALP activity by Zhang Mei-fen *et al.* (1997) [14] in canary bird. The oil red 'O' staining activity for fat was mild to moderate in the glandular units. However, the lipid material was reported to be abundant only in the gland cells of canary bird by Zhang Mei-fen *et al.* (1997) [14].

**5. Vagina**

The basal portion of the lining epithelium of vagina showed strong reaction for PAS and PAS - AB. However a weak to moderate PAS - AB reaction was reported in the vaginal epithelium by Ozen *et al.* (2009) [9] in duck and Bansal *et al.* (2010) [4] in Punjab white quail. The alkaline phosphatase activity was strong in the surface epithelial cells and the grooves in between the mucosal folds of the vagina, lamina propria, submucosal connective tissue (Fig. 6) and there was no acid phosphatase activity. However a strong ALP activity was reported to be observed only in the surface epithelial cells and grooves in between the folds by Bakst *et al.* (2007) in turkey. The oil red 'O' staining activity for fat was strong in the lining epithelium, while mild to moderate reaction was observed in the lamina propria and submucosa (Fig. 7).



**Fig 6:** Photomicrograph of the vagina showing strong alkaline phosphatase (ALP) (arrow) activity in the surface epithelial cells (E) between the mucosal folds, lamina propria (LP) and submucosal connective tissue (SM). ALP X 4



**Fig 7:** Photomicrograph of the vagina showing strong oil red 'O' (arrow) for fat in lining epithelium (E) and mild to moderate reaction in the lamina propria (LP) and submucosa (SM). Oil Red 'O' X 4

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