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HISTOCHEMICAL STUDY OF GLANDS OF OVIDUCT IN LAYING GEESE

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ABSTRACT

The present study was conducted on ten adult mature female geese to demonstrate the histochemical reaction of the glands of all the segments of the oviduct. The birds were slaughtered then dissected samples of different parts of oviduct were taken, fixed with 10% neutral buffered formalin, and 6 µm histological paraffin sections were stained by Hematoxylin and Eosin, Bromophenol blue, and Toluidine blue stain. The secretory units of neck glands gave a negative reaction with bromophenol blue stain and a positive reaction with Toluidine blue. The glands of magnum and isthmus were stained with Bromophenol blue and Toluidine blue. Some glandular cells of uterus gave a positive reaction with bromophenol blue stain but all the glandular epithelia gave a negative reaction with Toluidine blue. No glands were noticed in the wall of the vagina. The current study concluded that the main segments involve in egg protein formation are the magnum and isthmus. The vagina has no role in egg formation.

Keywords: Geese, oviduct, Bromophenol blue, egg protein

Introduction

The geese is very important in poultry industry, It has many biological features including the greatest growth intensity than chicken and other poultry species and has the ability to utilize green forages. The geese used in product meat, fat liver, goose fat and feathers. Goose meat that contains less moisture and high dry matter (Romanov, 1999). In some country the rising and breeding of geese is important in biosecurity and food safety (Prosser *et al.*; 2011). In avian species including the domestic birds, the female have only left ovary and oviduct the right one degenerate during early development (Yonju *et al.*; 2004; Abood and Al-Saffar, 2015).The avian oviduct is a highly complex and dynamic organ ,where the oocyte with the yolk which, released from the ovary is developed to the egg. During the egg production, oviduct tissues undergo extensive histological changes in lamina propria glands and secretary epithelial cells (Jeong *et al.*; 2012). All structural components of the laid egg are produced from the oviduct, except the yolk which formed from the ovum. The oviduct itself is a long tubular structure consisting of five functionally distinct parts (infundibulum, magnum, isthmus, shell gland or uterus, and vagina) (Rahman, 2013). After ovulation, the ovulated ovum is trapped by the action of the fimbriated region of the infundibulum. Regardless of fertilization, the ovum traverses through the oviduct. In the magnum, the albumen is deposited around the yolk, the shell membranes after that are produced in the isthmus, and the hard mineral shell is deposited in the uterus. In the vagina, the most caudal part, the cuticle is added to the egg prior to the oviposition segment. Albumin deposition, eggshell membranes formation, and shell calcification are genetically controlled (Willems *et al.*; 2014; Du *et al.*; 2015; Sah and Mishra, 2018). This study was designed to detect the glands involved in protein formation and deposition using histochemical stains bromophenol blue and Toluidine blue.

Materials and Methods

The current study was achieved on ten mature laying geese (Anser anser), aged (10-12) months. The birds were obtained from a local market in Baghdad city, Iraq, and brought to the anatomy laboratory of the College of Veterinary Medicine, University of Baghdad where the birds were humanely sacrificed. The celomic cavity was opened, the viscera removed to view the oviduct. The oviduct was dissected out and each oviduct segment was separated. Samples from each segment (infundibulum, magnum, isthmus, uterus, and vagina) were harvested, fixed in 10% Neutral buffered formalin for48 hours, and processed by paraffin embedding method. Paraffin sections at 6µm thickness were stained with Harris Hematoxylin and Eosin stain, for general histological structures of the oviduct; Bromophenol blue stain, for albumin detection in the oviduct glands; and Toluidine stain, for protein detection in the oviduct glands. Thereafter, stained slides were examined by a light microscope and photographed by a digital camera (model MC 500) with different magnifications (Bancroft and Cook, 1994).

Results and Discussion

The results of the current study showed that the wall of the female oviduct of geese consisted of four histological layers namely tunica mucosa, tunica submucosa, tunica muscularis, and serosa. The mucosa of all regions of oviduct formed mucosal folds, lined with pseudostratified columnar ciliated epithelium, lamina propria-submucosa contained branched tubular glands in all parts of oviduct except the funnel (infundibulum) and vagina the other part contain glands (Fig.1). The histological structures of oviduct segments of geese described in the current study were similar in almost all birds mentioned by previous studies such as in geese (Alshammary *et al.;* 2017), guinea fowl (Yoshimura and Ogawa,1998), chickens (Khokhlov, 2008), turkey hens (Mirhish and Nsaif, 2013), and duck (Essam *et al.*; 2016).

Glands of neck (Infundibulum)

The lamina propria submucosa of the funnel part involved loose connective tissue and highly enriched with blood vessels, tubular glands were also noticed in the neck only (Fig.1). In Toluidine blue stain histological section the cytoplasm of gland epithelial cells contained blue secretory granules (Fig. 2). The secretory units of neck glands gave a negative reaction with Bromophenol blue stain (Fig. 3). These results were in agreement with the results of Rahman *et al*. (2009) and Rahman (2013) in which they reported that the Chalaza and chalaziferous layer ropes of protein fibers consisted of protein secreted from the glandular cells of the neck region of the infundibulum. These results proved the results of Khokhlov (2008) and Essam *et al*. (2016) who recorded that the presence of tubular glands distributed within the connective tissue lamina propria of infundibulum in the oviduct of mature hen and Balady duck. Whereas Alshammary *et al*. (2017) found that the infundibulum in geese devoid of such glands. Patki *et al*. (2013) stated that the infundibulum glands were absent in immature Kuttanad duck.

Glands of Magnum

The glands of magnum occupied all the lamina propria submucosa, they were crowded, the connective tissue was hard to recognize. The glands were of simple branched tubular in type, the glandular epithelium characterized by pink cytoplasm and blue stained spherical nucleus in H&E stain (Fig.4). The glands cells cytoplasm contained blue secretory granules with Toluidine blue (Fig. 5). The secretory units of magnum glands gave a positive reaction with bromophenol blue stain, the cytoplasm stained dark blue (Fig.6).The shape and accordance of magnum gland were as described by Yoshimura and Ogawa(1998) in mature guinea fowl and chickens, Lucy and Harshan, (2000) in the adult Japanese quail, Mirhish and Nsaif (2013) in turkey hen, and Essam *et al*. (2016) in duck. They mentioned that the lamina propria of magnum was full of closely packaged well-developed branched tubular glands. In the present study, the positive reaction of glands cytoplasm with Bromophenol blue and Toluidine blue revealed the presence of a high concentration of albumin. This finding proved the results of Davidson (1986), Jung *et al*. (2011), and Alshammary *et al*. (2017). The authors reported that the magnum glands were the most interesting segment because it (the magnum) has a great role in the formation and deposition of white albumin around the yolk of the egg during it passing through the magnum.

Isthmus Glands

The lamina propria-submucosa of the isthmus contained numerous branched tubular glands similar to those of magnum. The glandular epithelia characterized by light pink cytoplasm and blue stained spherical nucleus with H&E stain (Fig.7). The glands epithelial cells cytoplasm contained blue secretory granules with Toluidine blue (Fig.8). glands gave a positive reaction with Bromophenol blue stain, the cytoplasm stained dark blue (Fig.9). The isthmus glands had the same structure as that of magnum. This observation was incomputable with the results of Balash and AL-Baghdady (2013) in turkey laying hen. The results of Essam *et al*. (2016) were different, however. They mentioned that the lamina propria submucosa of the isthmus of Balady female duck housed coiled tubular glands. The isthmus glands had the same histochemical reaction to Bromophenol blue and Toluidine blue as those of magnum, which indicates that the glands produce protein. This finding was in agreement with the results of Alshammary *et al*. (2017) in geese, where the authors mentioned that the glands involved in secreting and formation of the shell membranes consisted mainly of protein.

Glands of Uterus

The lamina propria submucosa consisted of vascularized loose connective tissue separates between the branched tubular glands. The glands were less occurrence than that of magnum and isthmus, and the gland epithelial cells was pale stained in some cells and pink in another H&E stain (Fig.10).all the glandular epithelia cells gave a negative reaction with Toluidine blue (Fig. 11). The same observation was noticed in the case of Bromophenol blue stain, put few cells gave a positive reaction (Fig. 12), The presence of few branched tubular glands in lamina propria-submucosa of the uterus was similar to that mentioned by Yoshimura and Ogawa (1998) in guinea fowl and chickens and the results of Essam *et al*. (2016) in mature Balady duck female. The histochemical results of the current study revealed that some glandular cells are involved in protein recreation. This result was in parallel with the results of Kusuda *et al.* (2011) and Samiullah and Roberts(2014), they found that the eggshell in all birds consisted of Ca- carbonates, Ca- phosphorous, and cuticle.

The Vagina

The lamina propria submucosa of the vagina lacked tubular glands (Fig.13, 14). The tunica muscularis of vaginal wall was thick and well developed, particularly the circular layer. The absence of gland in the lamina propria submucosa was mentioned and reported by Lucy and Harshan (2000) in the adult Japanese quail, El-Zoghby *et al.* (2014) in the Egyptian Geese, and Essam *et al.* (2016). in the duck. Because of the absence of gland and the presence of thick muscular of the vaginal wall and a well developed muscular sphincter at the uterine-vaginal, the current study suggests that the vagina in geese did not have any role in egg formation, but expel the egg out of the geese body. This fact was in agreement with the results of Alshammary *et al.* (2017) in geese and the results of Rahman (2013).

Fig. 1 : Histological section (neck) shows epithelium (E), lamina propria (Lp). Toluidine blue stain, 100x

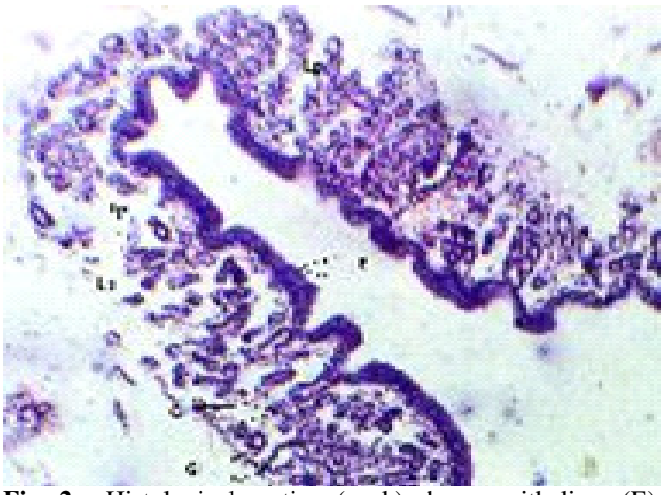

Fig. 2 : Histological section (neck) shows epithelium (E), lamin propria (Lp), and glands (G). H&E stain, 40x

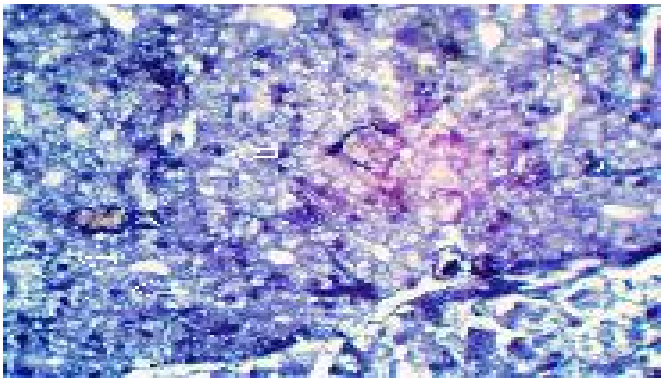

Fig. 3 : Histological section (neck) shows that the gland cells did not stain (white arrows). Bromophenole blue stain, 100x)

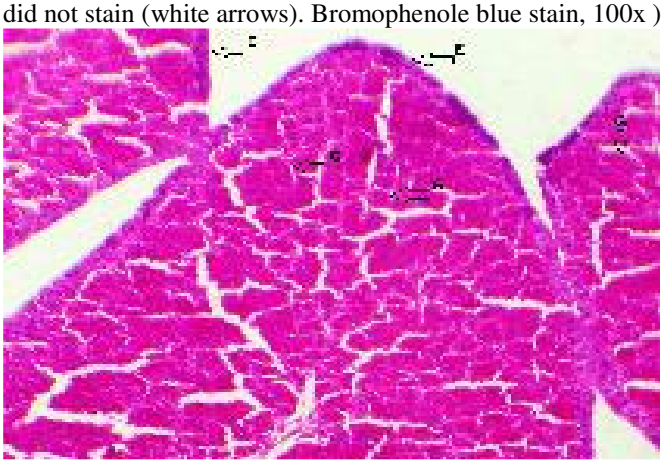

Fig. 4 : Histological section of magnum shows epithelium (E), lamina propria (Lp), glands (G). H&E stain, 40x

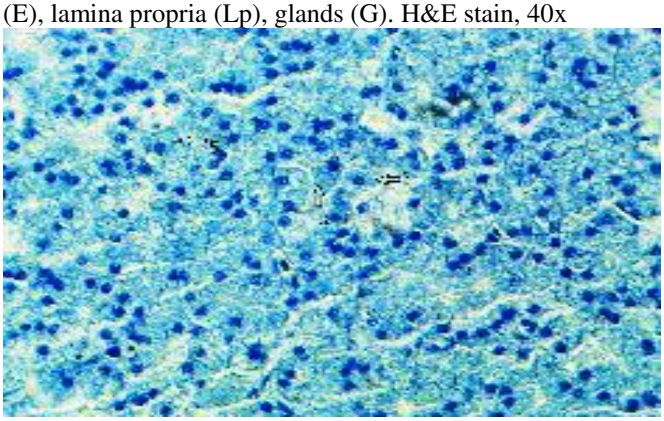

Fig. 5 : Histological section of magnum shows blue secretory granules (S). Toluidine blue stain, 100x

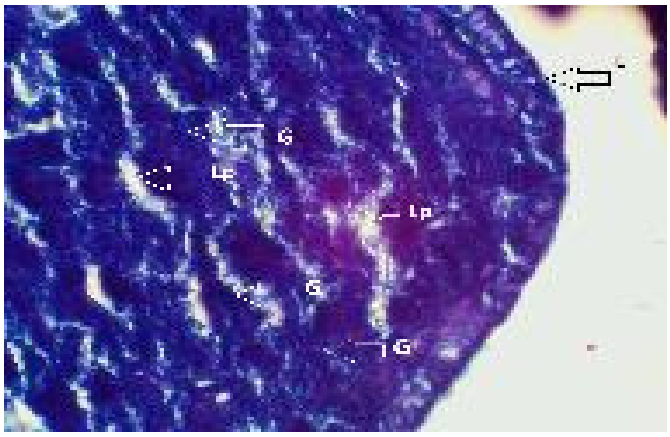

Fig. 6 : Histological section of magnum shows epithelia (E),lamina propria (Lp), gland (G) stained dark blue. Bromophenol blue stain, 100x)

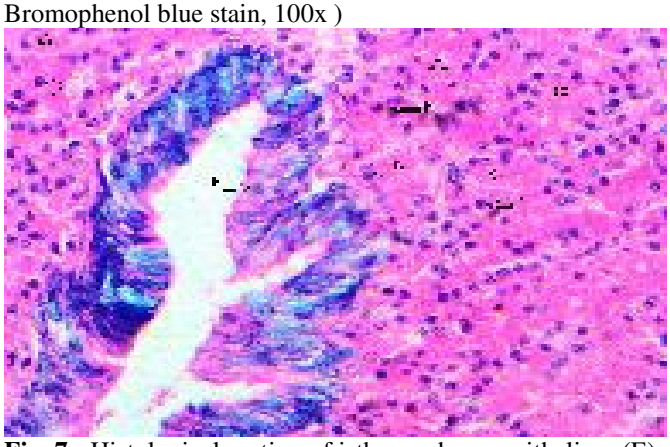

Fig. 7 : Histological section of isthmus shows epithelium (E), nucleus (N), .cytoplasm(stars). H&E stain, 100x

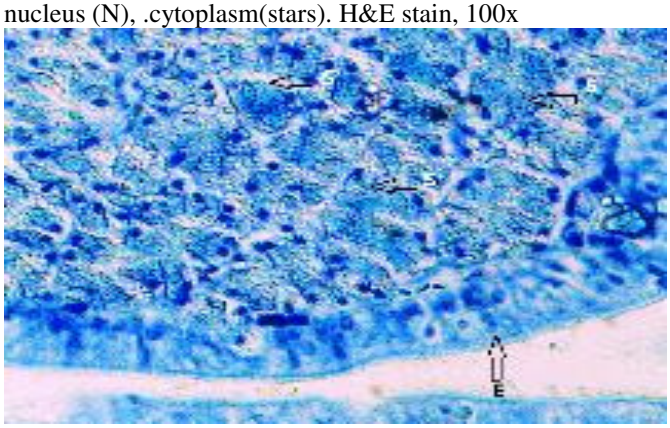

Fig. 8 : Histological section of isthmus shows blue secretory granules (S), epithelia (E). Toluidine blue stain, 100x

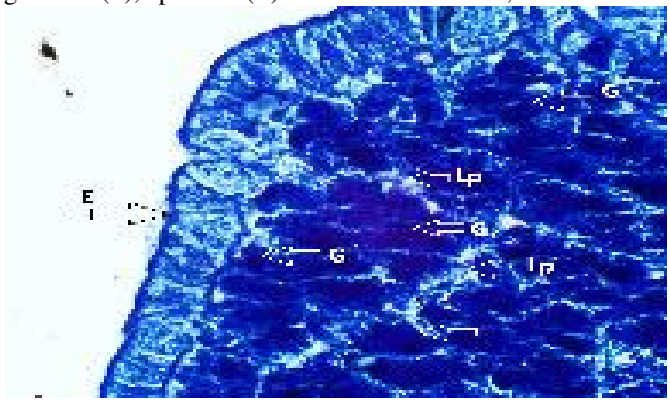

Fig. 9 : Histological section of isthmus shows epithelium (E), lamina propria (Lp), glands (G) stained dark blue. Bromophenol blue stain,100x

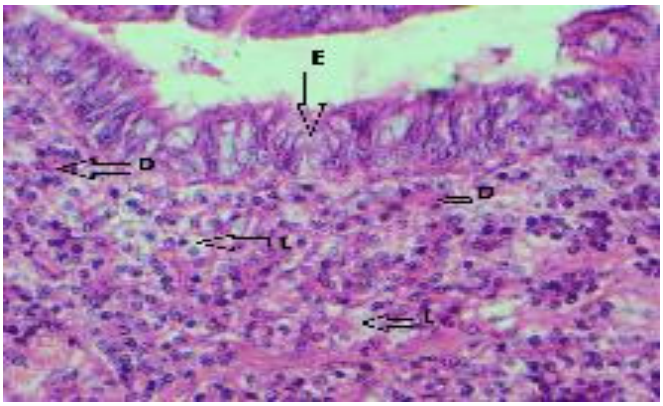

Fig. 10 : Histological section of uterus shows epithelium (E), pale stain cytoplasm (L), dark stain cytoplasm (D). H&E stain,100x

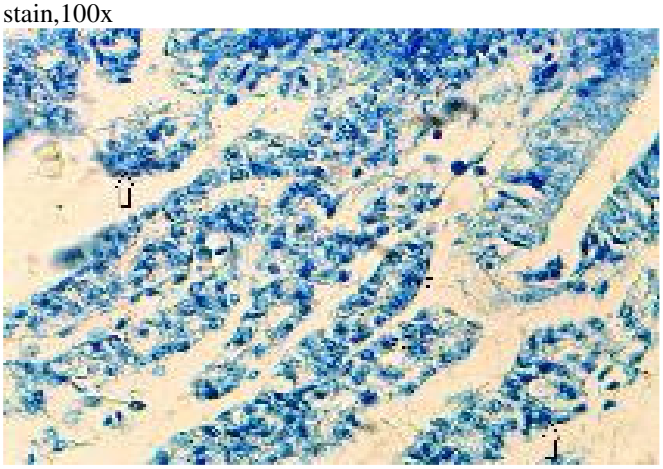

Fig. 11 : Histological section of uterus shows blue secretory granules (S) (arrows). Toluidine blue stain, 100x

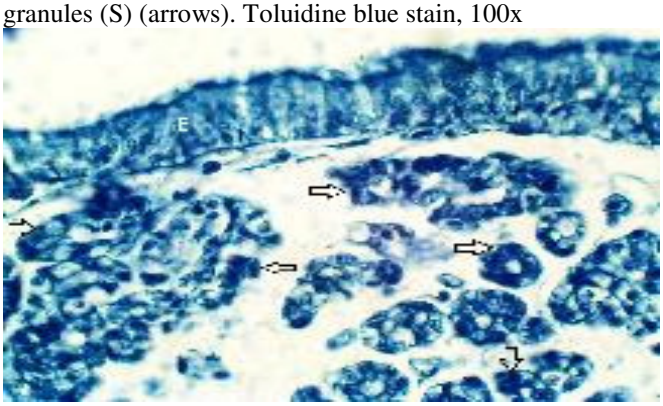

Fig. 12 : Histological section of uterus shows epithelia (E), some gland cells stained dark blue (arrows). Bromophenol blue stain, 400x

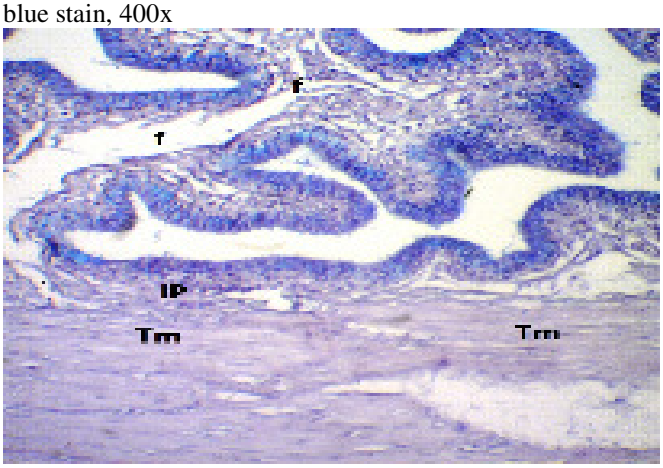

Fig. 13 : Histological section of vagina shows lamina propria (Lp) (glandless), tunica muscularis (Tm). H&E stain, 40x)

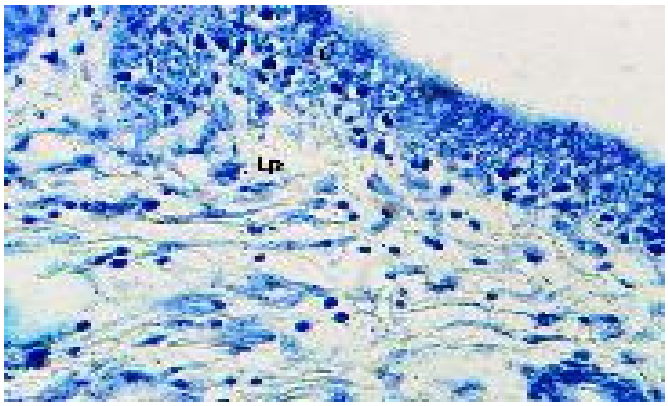

Fig. 14 : Histological section of vagina shows lamina propria (Lp) (gland less), tunica muscularis (Tm). Bromophenole blue stain, 100x

References

Abood, D.A. and Al-Saffar, F.J. (2015). The post hatching development of the female genital system in Indigenous Mallard Duck (*Anas platyrhynchos*). The Iraqi Journal of Veterinary Medicine, 39(2): 17-25.

Alshammary, H.K.A.; Jabar, A.I. and Nasser, R.A. (2017). Geese ovary and oviduct from an Anatomical and Histological point of view. Research Journal of Pharmaceutical, Biological and Chemical Sciences, 8(6): 207- 219.

Balash, Kh. J. and AL-Baghdady, E.F. (2013). Histological Study of the Isthmus Segment of the Oviduct in Female Turkey at Egg-Laying Stage. AL-Qadisiya Journal of Vet. Med. Sci.; 12(2): 13-18.

Bancroft, J.D. and Cook, H.C. (1994). Manual of Histological Techniques and their diagnostic application. Longman group U.K. London Madrid.

Davidson, M.F. (1986). Histological studies of changes in the magnum of the domestic hen associated with the production of 'watery white' eggs. Br Poult Sci.; 27(2): 353-354.

Du, J.; Hincke, M.T.; Rose-Martel, M.; Hennequet-Antier, C.; Brionne, A.; Cogburn, L.A.; Nys, Y. and Gautron, J. (2015) Identifying specific proteins involved in eggshell membrane formation using gene expression analysis and bioinformatics. BMC Genomics 16: 1-13.

El-Zoghby, I.M.; Reda, IE. and Abdelfattah, N. (2014). Some Histological, Histochemical, and Ultra Structure Studies on The Vagina of The Egyptian Geese. Benha Veterinary Medical Journal, 26(1): 113-119.

Essam, M.E.L.; Asmaa, M.I.; Samah, H.El.; Shaker, N.A. and Shaimaa, H.H. (2016). Morphological and Histological Studies on the Female Oviduct of Balady Duck (*Anas boschas domesticus*). International Journal of Advanced Research in Biological Sciences, 3(7): 171-180.

Jeong, W.; Lim, W.; Kim, J.; Ahn, S.E.; Lee, H.C.; Jeong, J.-W.; Han, J.Y.; Song, G. and Bazer, F.W. (2012). Cell-specific and temporal aspects of gene expression in the chicken oviduct at different stages of the laying cycle. Biology of Reproduction 86: 172.

Khokhlov, R.Y. (2008). Morphology of an Infundibulum of the Oviduct of the Sexually Mature Hens. Int. J. Morphol.; 26(4): 883-886.

Kusuda, S.; Iwasawa, A.; Doi, O.; Ohya, Y. and Yoshizaki, N. (2011). Diversity of Eggshell Cuticle Journal of Poultry Science, 48 (2): 119-124.

Lucy, K.M. and Harshan, K.R. (2000). Structure and Postnatal Development of Magnum In Japanese Quail. Journal of Veterinary and Animal Sciences, 31: 40-43.

Mirhish, S.M. and Nsaif, R.H. (2013). Histological Study of the Magnum and Vagina in Turkey Hens Meleagris gallopavo. G.J.B.B.2 (3): 382-385.

Patki, H.S; Lucy, K.M. and Chungath, J.J. (2013). Histological Observations on the Infundibulum of Kuttanad Duck (Anas platyrhynchos domestics) during Postnatal Period International Journal of Scientific and Research Publications, 3(1): 1-8.

Prosser, D.J.; Wu, J.; Ellis, E.C.; Gale, F.; Van Boeckel, T.P.; Wint, W.; Robinson, T.; Xiao, X. and Gilbert, M. (2011). Modelling the distribution of chickens, ducks, and geese in China. Agric Ecosyst Environ, 1; 141(3-4): 381–389.

Rahman, M.A; Moriyama, A.; Iwasawa, A. and Yoshizaki, N. (2009). VMO-II mediates the binding of the chalaziferous layer with the vitelline membrane in quail eggs. J. Pout. Sci. 46: 240-248.

Rahman, M.A. (2013). An Introduction To Morphology Of The Reproductive System And Anatomy of Hen's Egg . J. Life Earth Sci., 8: 1-10.

Romanov, M.N. (1999). Goose production efficiency as influenced by genotype, nutrition and production systems" Worlds Poultry Science Journal, 55: 281-291.

Sah, N. and Mishra, B. (2018). Regulation of egg formation in the oviduct of laying hen . World's Poultry Science Journal, 74: 1-13.

Samiullah, S. and Roberts, J.R. (2014). The eggshell cuticle of the laying hen. World's Poultry Science Journal, 70: 693-708.

Yonju, H.T.; Akira, S.; Noboru and Kiyoshi, S. (2004). Changes in mRNA expression of the MMP-2 in the Mullerian duct of the chicken embryo. Gen. Comp. Endocrinol.; 139: 131-136.

Yoshimura, Y. and Ogawa, H. (1998). Histological Characterization of the Oviducal Structures in Guinea Fowl (*Numida meleagris*). Jpn. Poult. Sci.; 35: 149-156.

Willems, E.; Decuypere, E.; Buyse, J. and Everaert, N. (2014). Importance of albumen during embryonic development in avian species, with emphasis on domestic chicken. World's Poultry Science Journal 70: 503-518.