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Histological and Histochemical study of upper third of esophagus in young rabbit: in AL-Muthanna

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Abstract

The current study was carried out at the College of the Veterinary Medicine- of Al-Muthanna University for observing Histological and histochemical in The esophagus of young rabbits with a focus on regional structural differences across the cervical segment. The esophagus is composed of four main layers: the tunica mucosa, the submucosa , the muscularis externa, and the tunica adventitia. The mucosal layer is made up of a stratified squamous epithelium that exhibits partial keratinization, which is especially noticeable in the cervical region, in contrast to the non-keratinized epithelium seen in humans and cats. The lamina propria contains dense connective tissue that is rich in small and medium blood vessels, while the muscularis mucosa is composed of discontinuous longitudinal bundles of smooth muscle. The submucosa is highly vascularized and does not have esophageal glands, which is different from certain other mammals and birds. Three separate layers of striated muscle fibers make up the complete muscularis externa. The adventitia, a vascularized loose connective tissue covering the outer surface of the esophagus, is about 2760 ±24 µm thick.

Keywords**: **esophagus, rabbit , histology, Histochemical

I. INTRODUCTION

The closest evolutionary related to humans, after primates, is the rabbit (Oryctolagus cuniculus), a member of the Lagomorpha group. In terms of husbandry, ease of breeding, cost-effectiveness, and ethical and legal conveniences, it is a more acceptable laboratory mammal than primates. Furthermore, compared to mice or rats, rabbits have advantages as laboratory animals due to their phylogenetic similarity to humans, size, blood volume, responsiveness, and other congruences that allow them to more accurately mimic human physiological traits in biomedical research (Dutta & Sengupta, 2018). Worldwide, rabbits are regarded as a significant and healthful source of animal protein (Abd El-Ghany, 2020). The rabbit classification based on per (Mojari and Saluqi 2013).

Domain: *Eukaryota*
Kingdom: *Animalia*

Phylum: *Chordata*

Class: *Mammalia*

Order: *Lagomorpha*

Family: *Leporidae*

Genus: *Oryctolagus*

Species: *Oryctolagus cuniculus*

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From the pharynx to the stomach's cardia, the esophagus is a straight extension of the pharynx. Up until it entered the stomach, the esophagus was dorsal to the trachea. The esophagus is divided into three portions based on where it is located in the body: the cervical, thoracic, and abdominal (Kadhim, 2019; Imam et al., 2021).

II. MATERIAL AND METHOD

Collection of specimens:

The current study was carried out at the University of Al-Muthanna's College of Veterinary Medicine, Department of Anatomy, Histology, and Embryology. September 15, 2024, to June 1, 2025, was the study period. There were: 10 young rabbits .The rabbits' weights ranged from 150 to 250 gm, The animals were gathered from nearby markets, and each one underwent a clinical test to make sure it was healthy.

Surgical Procedures ,Histological and histochemical study:

Two milliliters of chloroform ($CHCl_3$) were applied to cotton and placed close to the animal's nose to anesthetize it before euthanasia (Besoluk et al., 2006). The rabbits' abdomens were then carefully cut along their length to remove the esophagus using the proper instruments, including scissors, forceps, and dissection scalpels (Bennett,2021and Acosta,et al.,2021).
Following anesthesia, each animal's esophagus were removed, and the cavities were cleaned with running water. The samples were taken from the esophagus, from the cervical regions. Following that, the standard protocol for histological preparation was followed (Isaac et al., 2023).

Sample Fixation

Following extraction, the esophageal specimens—which were meant for histological analysis—were preserved in a 10% formalin solution .The duration of the fixation procedure was 48 hours. To get rid of the formalin solution, the specimens were rinsed with tap water for two to three hours after fixing.

Dehydration:

For two hours at each concentration, the specimens were exposed to increasing ethyl alcohol concentrations, beginning with 70%, 80%, 90%, 100%, and 100%. This was done in an effort to progressively eliminate the water that was inside the tissue.

Clearing:

To increase their transparency and get rid of the alcohol solution, the specimens were treated with xylene twice for an hour each.

Infiltration:

Following cleaning, the samples were placed in glass containers with a 1:1 melted paraffin wax and xylene combination in an electric oven that was heated to 59–60°C. After that, the specimens were moved to different containers that contained solely melted paraffin wax. To guarantee that the specimens were completely impregnated, the wax was changed twice, with a one to one and a half-hour interval between changes.

Embedding:

Paraffin wax was used to place the specimens in specialized metal molds. To eliminate any air bubbles surrounding the specimens, a heated needle was placed over a flame. After allowing the molds to solidify at room temperature, the specimens were taken out of the mold and kept in storage until they were cut into sections.

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Trimming and Sectioning:

A sharp scalpel was used to trim the paraffin blocks that held the specimens. The specimens were then sliced to a thickness of 5 micrometers after the paraffin block had been mounted for sectioning in a Rotary Microtome. The cut ribbons were transferred to a water bath maintained at 45°C to promote optimal tissue spreading. The tissue pieces were then placed on clean glass slides, left to dry on a hot plate at 37°C for one hour, then kept at room temperature until the following day.

Staining:

Using a variety of stains, including Masson's trichrome, Alcian blue, and Periodic acid Schiff (PAS), (Suvarna,et al.,2018) describe the histochemical characteristics of the cells in each section of the stomach and esophagus.

Eosin and Mayer Haematoxylin Stain(H&E): It was a standard dye used in histological investigations to illustrate the tissue's overall structure
Masson Trichrome Stain: The purpose of this stain is to show how smooth muscle and connective tissue fibers appear

Periodic Acid-Schiff (PAS) Stain: (Bancroft and stevens, 2018) : This stain was utilized to demonstrate of neutral muco-polysaccharide secretion
Alcian Blue stain :Acid muco-polysaccharide secretion was demonstrated

Mounting: The sections are placed on a glass slide that contains a 1:1 ratio of Mayer's albumin (a mixture of egg albumin and glycerin). Slides were dried on a heated plate at 40ºC for 24 hours after a few drops of thymul were added to stop degradation (the growth of bacteria and fungus)

Histological measurements: Using a light microscope and an ocular micrometer, histological measurements were made of every part of the esophagus. These measurements included the thickness of the tunica mucosa (epithelium, lamina propria, and muscularis mucosa), the tunica submucosa, the tunica muscularis (circular and longitudinal layers), and the tunica adventitia of each section.

statistical analysis:

The mean values with the standard error (SE) have been used to report the numerical,results.

III. RESULTS AND DISCUSSION

According to the histological findings, the young rabbit's esophagus is composed of four layers from the inside out: tunica mucosa, tunica submucosa, muscularis externia and tunica adventitia in the cervical and thoracic regions, and tunica serosa in the abdominal region. These layers are similar to those found in other mammals (Konig & Liebich, 2020). The lumen has a characteristic star-like appearance due to the longitudinal folds formed by the mucosa and submucosa along the esophagus while at rest. These folds are lessened as the esophagus lumen increases during food passage(Figure1,2).

Muscularis mucosa, lamina propria, and epithelium make up the mucosa. The squamous stratified epithelium had a propensity for keratinization, which is more pronounced in the cervical segment than in the other two. This outcome is different from what has been seen in people and cats (Raica et al., 2004), where it was of the non-keratinized variety. According to (Ranjan and Das 2016), in the cervical section.

Numerous small and medium-sized blood vessels are found in the lamina propria, which is made up of connective tissue that is comparatively dense toward the epithelium and clearly loose toward the muscularis mucosa (Figure1).

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Although it is there, the muscularis mucosae does not provide a continuous layer of muscle. It is composed of longitudinally oriented, discontinuous bundles of smooth muscle cells spaced a specific distance apart. Different esophageal segments have different muscle bundle densities and numbers. The cervical region of the esophagus has the fewest muscular,bundles(Figure2).

The submucosa is made up of loose connective tissue that is highly vascularized. This tissue allows the mucosa to slide over the muscularis externa during the large volume changes that the esophagus experiences from rest to food bowl passage. It should be mentioned that, unlike other animals like dogs (Rus et al., 2016) and storks (Rus et al., 2009), we could not find esophageal glands in the submucosa in any of the segments in this species(Figure2).

An exterior longitudinal layer, a medium circular layer, and an internal longitudinal layer are the three layers of striated muscle fibers that make up the rabbit esophageal muscularis externa. In the cervical region, the exterior longitudinal muscle layer is the thinnest (Figure1). Adventitia, which is made up of loose, well-vascularized connective tissue, coated the outside of the esophagus. In average, its thickness is 2760 ±24 µm (Table1)

Study, both PAS (periodic acid-Schiff) and Alcian blue stains were utilized. The study showed that both stains reacted positively with the tissue, indicating the presence of carbohydrates. It was also observed that there are no glands in the cervical part of the rabbit's esophagus(Figure3,4).

Table (1). Show histomorphometric parameter of Esophagus in in Young

Rabbit Values represent **: µm (Mean ± SE).**

Tunica	**Mean ± SE**
total Mucosa	1090.667 ±55
Keratine	300 ±22
epithelium	610.667 ± 31
Lamina propria	90 ±11
Muscularis mucosae	90 ±12
total Submucosa	503 ±22
total Muscularis	2990 ±11
internal	90 ±10
middle	1900 ±22
external	1000 ±21
Adventitia	2760 ±24

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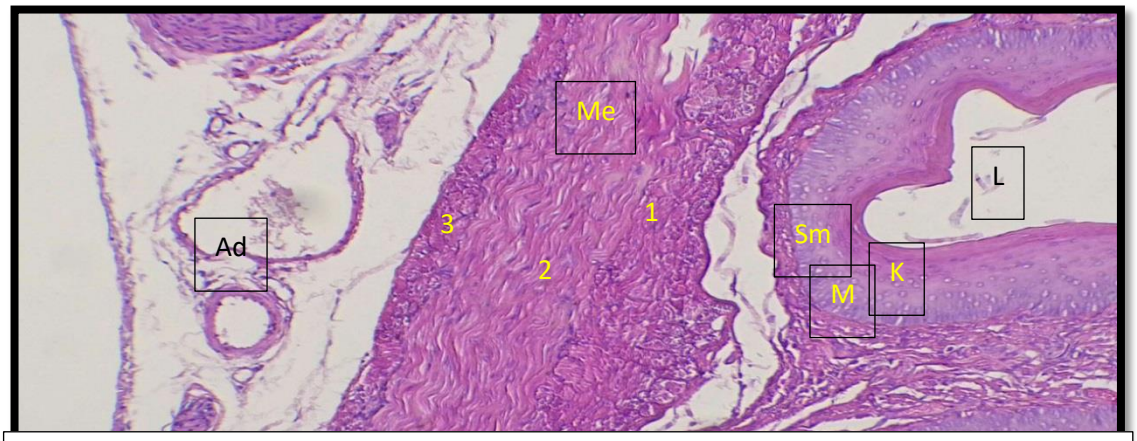

Figure 1. Histological cross section of the cervical esophagus in young rabbit shows: (L) Lumen, (K) keratin , (M) mucosa , (Sm) submucosa , (Me) muscularis(1- internal layer 2- middle layer3- external layer), (Ad) adventitia **H&E stain.10X**

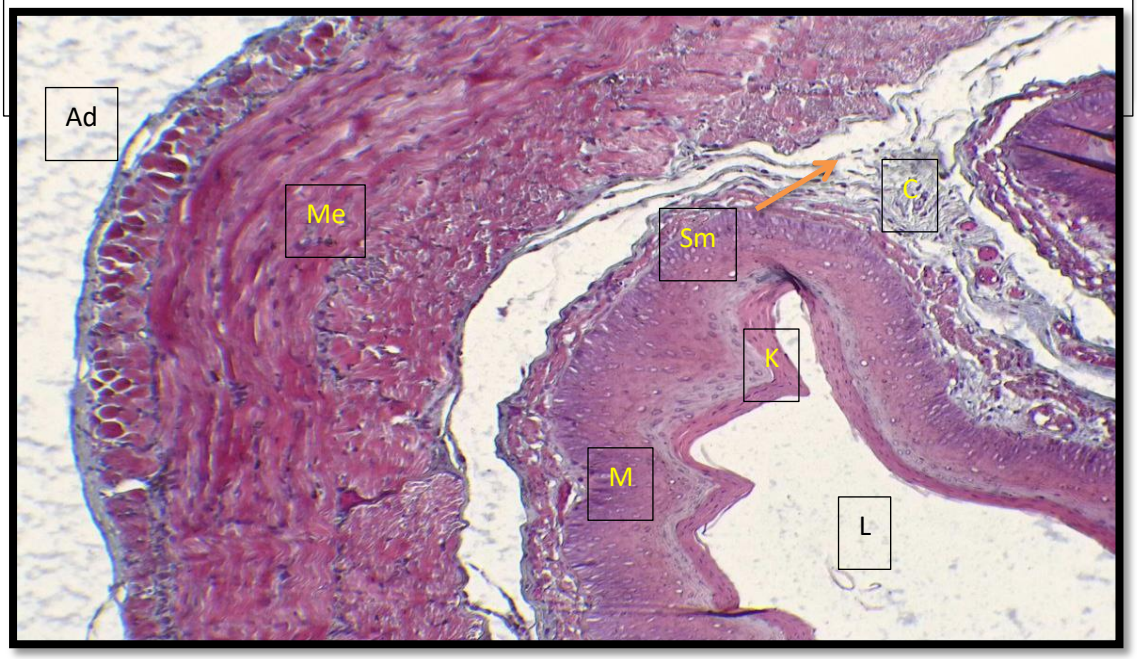

Figure 2. Histological cross section of the cervical esophagus in young rabbit shows: (L) Lumen, (K) keratin , (M) mucosa , (Sm) submucosa , (Me) muscularis, (Ad) adventitia, (C) collagen fibres **Masson's Trichrome stain. X 4**

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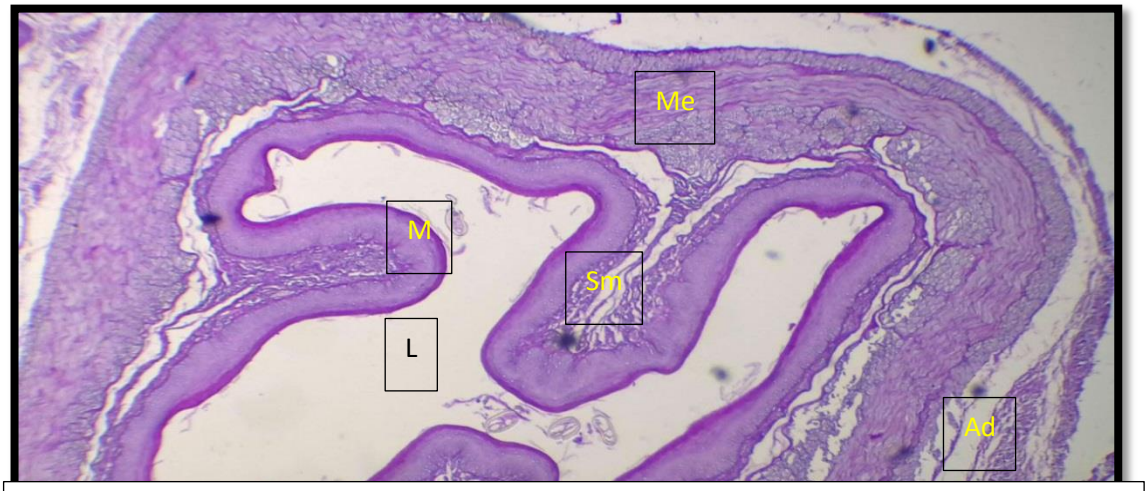

Figure 3. Histological cross section of the cervical esophagus in young rabbit shows: (L) Lumen, (M) mucosa , (Sm) submucosa , (Me) muscualaris, (Ad) adventitia No esophageal glands **X4 PAS stain.**

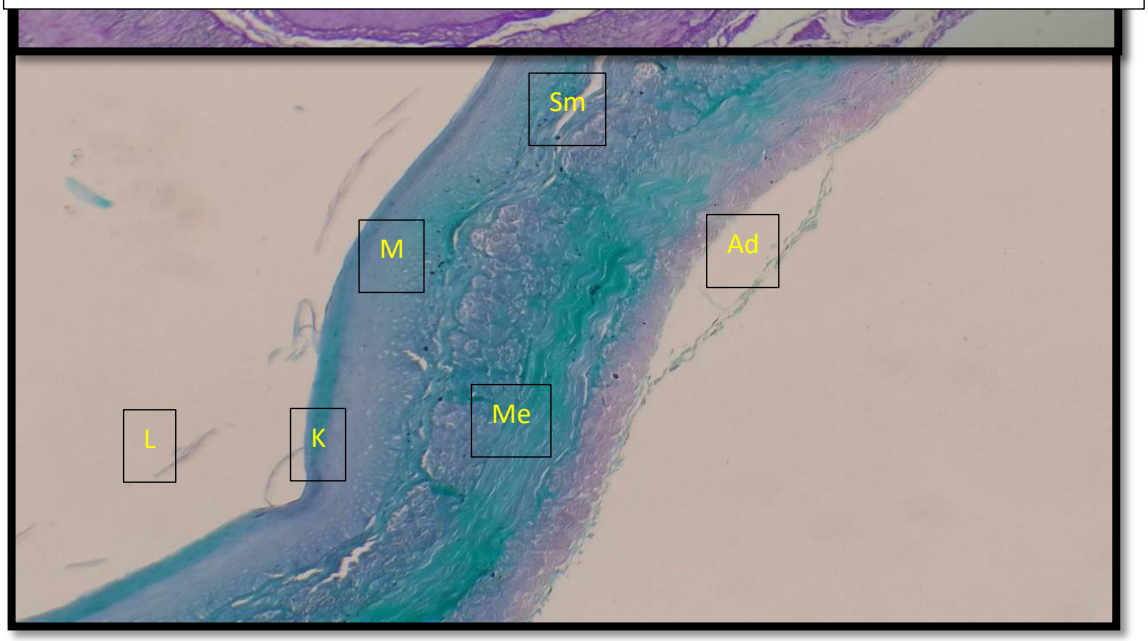

Figure 4. Histological cross section of the cervical esophagus in young rabbit shows: (L) Lumen, (K) keratin , (M) mucosa , (Sm) submucosa , (Me) muscualaris, (Ad) adventitia No esophageal glands **X4 Alcian Blue stain.**

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IV. CONCLUSIONS

The esophagus of adult rabbit exhibits a complex, regionally varied histological architecture that reflects both species-specific adaptations and universal mammalian patterns. Notable characteristics that set the rabbit esophagus apart from other species include the presence of keratinized stratified squamous epithelium in the cervical area, the lack of esophageal glands in the submucosa, and the distinct arrangement of muscle layers in the muscularis externa. These traits point to dietary and feeding-related functional changes. The study's conclusions offer useful baseline information for upcoming developmental and comparative histology investigations.

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