

Histological and histochemical studies on the structural development of the trachea in Awassi rams *Ovis aries* during postnatal and sucking life

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Abstract

The study's objective was to describe the trachea's histological and histochemical characteristics at various ages in Awassi rams. The study was conducted on (14) samples of the trachea parts of healthy local rams; One day old as newly born lambs (7) samples and Forty day old as suckling lambs (7) samples. Five samples were taken from each region of the cervical and thoracic parts of the trachea and fixed in 10% formalin for 24 hours before being processed using standard histological techniques and the sections stained with PAS, PAS-AB, and Masson's Trichrome. The mucosa, submucosa, hyaline cartilage, muscle, and adventitia make up the trachea's wall. A pseudostratified ciliated columnar epithelium, consisted of the three major cells (ciliated columnar cells, basal cells, and goblet cells), lined the tracheal mucosa. The percentage of the goblet cells in the trachea of the suckling ages was higher than that of newly born ages and their number in cervical part of trachea was more than that in thoracic part in all studies lambs. The goblet cells responded favorably to every periodic acid Schiff and to Alcian blue-periodic acid Schiff, the lamina propria-submucosa was composed of loose connective tissue with elastic fibers, blood vessels, and alveolar mucous glands in newly born lambs, while in suckling lambs, mixed glands were present. The cervical part of the tracheal epithelium contains a large number of simple alveolar mucous glands, while in the thoracic part of the epithelium, the mucous glands were lesser and replaced in some regions by goblet cells which forms 'intraepithelial glands. The posterior ends of the cartilages were joined by dense fibroelastic tissues and smooth muscles, and the incomplete cartilaginous rings were flattened and hyaline, surrounded by the perichondrium. The cartilage was made up of the ground-up chondrocytes. The cartilaginous rings were coated in a thin tunica adventitia of the loose connective tissue, and the trachealis muscle was made up of elastic fibers and smooth fibers. Lambs in the sucking age group had mucosa, submucosa, and adventitia that were thicker than those in lambs that had just been born, as well as more goblet cells overall than lambs that had just been born. In conclusion, the trachea of lambs of the various ages varied in the goblet cells quantity and type as well as histological layer thickness.

Keywords: *Awassi rams, lambs, Trachea, Histological.*

INTRODUCTION

In addition to facilitating the conduction and exchange of gases, the respiratory system also regulates body temperature, produces and excretes a variety of substances, including histamine, and aids in the creation of the voice. and connected to the olfactory system (1,2). The respiratory system includes; the nostrils, nasal cavity, nasopharynx, trachea, bronchi, bronchioles, and lungs (3). Both within and between species, there are variations in the respiratory tract architecture. The dog, common rodents, and rabbits have primarily monopodial branching systems, whereas human and non-human primate lung have irregular dichotomous and Trichotomus air route branching patterns (1,4). The trachea is a tube-like structure that connects to the caudal border of the laryngeal cartilage and is located on the ventral side of the neck. It extends from a transverse plane through the middle of the axis to a plane between the fourth and fifth thoracic vertebrae and its made up of different numbers of c-shaped cartilages depending on the species (5-8).

The trachea is divided into two segments depending on the position. The cervical trachea is lateralized by the longus muscle and is restricted ventrally and bilaterally by the stone-thyrohyoidus muscle (9). The pseudostratified ciliated colimmar epithelium that lined the trachea contained ciliated, basal, and goblet cells (10). (11) mention respiratory epithelium, goblet cells are the primary secretory cell type in the tracheal epithelium of domestic mammals; Clara cells in other species; submucosal glands; and trachealis muscle to narrow the tracheal lumen. Hyaline cartilage is used to make the tracheal rings, which are crucial for supporting the trachea's structure (12-14). There are few articles available on trachea developmental histomorphological study

of the lambs in southern Iraq; The aim of the present study to focused the light on the features of the lambs trachea, these Knowledge was essential for provides baseline data for investigative researches and helps in understanding the physiology, pathology and further sciences.

Materials and method

The study was performed using Ten of the each healthy local breeds Awassi rams, One day age as newly born lambs (7) samples and Forty day age as suckling lambs (7) samples, were collected from AL-Muthanaa abattoir during January and February 2022, the samples were taken from the different parts of the (cervical and thoracic parts of trachea), five specimens was taken from each region and fixed in 10% formalin for 24 hours, then treated by routine histological technique, the stains that used; Harris hematoxylin and Eosin (H&E) stain; For demonstrating the general histological components of the tissue, Periodic acid/Schiff (PAS) stain; To the mucoprotein, carbohydrate, mucopolysaccharides, glycoprotein, and basement membrane, Masson's trichrome stain; To demonstrate the collagen and the smooth muscles (15). The

thickness of the mucosa, submucosa and adventitia tunicae of each part of

trachea in each age, in five sections in each section of each part of the trachea by ocular micrometer. goblet cells counts, ten sections from each part of the trachea, in each section the cells in ten microscopic fields (Per microscopic field (X200) were counted. Mean (\bar{x}) \pm standard error (S E) were calculated for each the measurement, thirty slides of the each part (16).

Results

The mucosa, submucosa, muscularis, and adventitia tunicae make up the trachea's wall four layers, which were cartilaginous and not collapsible tube. The mucosal lining was made up of respiratory epithelium and many goblet cells that secrete mucus and rest on a basement membrane; this epithelium was surrounded by the lamina propria-submucosa which composed of the loose connective tissue and contains; capillaries, lymphocytes, longitudinally arrangement of elastic fibers and blood vessels, and well developed subtending adventitia with incomplete cartilaginous rings (Fig. 1,2). In a uniform matrix, the chondrocytes were present in clusters, when exposed to PAS, the cartilage matrix reacted strongly; chondrocytes, on the other hand, reacted weakly, and the perichondrium reacted strongly (Fig. 3). While with PAS-AB stain the cartilage matrix, chondrocytes and perichondrium reacted moderately (Fig. 1,2),.

A large portion of the tracheal epithelium was made up of tall, columnar ciliated, goblet cell, and basal cells (Fig. 4,5). The trachea's length was covered in goblet cells, which were scattered irregularly amid the columnar cells, involved in mucus production, between the other cells were wedged basal cells (Fig. 4). The ciliated cells were completely in trachea of suckling lambs, while was not found in some part of thoracic portion of trachea of new born lambs (Fig.2,4,7) and the epithelium of all trachea parts in suckling lambs is thicker, has a higher cells density and rests on a thicker basement membrane than that described for newborn lambs trachea (Fig.2,4). The ventral region of the trachea has a higher concentration of tracheal glands than the dorsal portion (Fig.4-6), these glands were take positive reaction with (PAS) and with PAS-AB (Fig.1,3,8), mucous glands in newly born

lambs; while mixed glands in suckling lambs, Numerous simple alveolar mucous glands can be seen in the cervical region of the tracheal epithelium, while in thoracic part was found lesser number from the mucous glands and most regions of this part has the goblet cells, which create "intraepithelial glands," supplanted the mucous glands in the cervical epithelium, and these glands were less developed in cervical trachea than that in thoracic trachea in each ages (Fig. 1,8), the number of goblet cells in cervical and thoracic parts of trachea in newborn lamb was $(40 \pm 2, 35 \pm 1)$, while in suckling lambs was $(48 \pm 1, 41 \pm 2)$ and their number in cervical part of trachea more than that in thoracic part, thickness of mucosa in cervical trachea in newborn lamb was $(62.2 \pm 1.1 \mu\text{m})$, while in suckling lambs was $(74.6 \pm 1.3 \mu\text{m})$, and it thickness in thoracic trachea in newborn lamb was $(58.3 \pm 1.6 \mu\text{m})$, while in suckling lambs was $(70.1 \pm 1.2 \mu\text{m})$, and mean thickness of mucosa in the cervical trachea was thicker than that in the thoracic trachea of each study animals (Table 1). Thickness of submucosa in cervical and thoracic parts of the trachea in newborn lamb was $(247.6 \pm 1.2, 232.3 \pm 2.1 \mu\text{m})$ respectively, while in suckling lambs trachea was $(295.2 \pm 2.3, 280.2 \pm 1.6 \mu\text{m})$ respectively (Table 1). The thickness of the mucosa and submucosa was gradually decreased towards the thoracic part of the trachea (Table 1).

The free ends of the cartilaginous crescents are connected by interlacing bundles of the smooth fibers by the trachealis muscle, which was located on the posterior membranous section of the trachea. The posterior trachea wall of rams lacks the cartilage and maintained by a thin bands of smooth muscles (Fig.7). Thickness of adventitia in cervical and thoracic parts of trachea in newborn lamb was $(71.3 \pm 0.4, 74.5 \pm 0.3 \mu\text{m})$ respectively, while in

suckling lambs trachea was (78.2 ± 0.2 , 81.6 ± 0.1 μm) respectively (Table 1).

Table(1) :Measurement of thickness of the wall layers and number of goblet cells (Per microscopic field (X200)) in trachea of Awassi rams (μm) (X- \pm S.E)

Part Measure	Tunica mucosa	Tunica submucosa	Tunica adventitia	Number of goblet cells
Cervical part in newborn lambs In suckling lambs	62.2 ± 1.1	247.6 ± 1.2	71.3 ± 0.4	40 ± 2
	74.6 ± 1.3	295.2 ± 2.3	78.2 ± 0.2	48 ± 1
Thoracic part in newborn lambs In suckling lambs	58.3 ± 1.6	232.3 ± 2.1	74.5 ± 0.3	35 ± 1
	70.1 ± 1.2	280.2 ± 1.6	81.6 ± 0.1	41 ± 2

Fig.(1): Cross section of the cervical part of newly born lamb trachea; mucosa (A), submucosa (B), cartilage (C), smooth muscle (D), adventitia (E), lumen of esophagus (F), PAS-AB, 40X

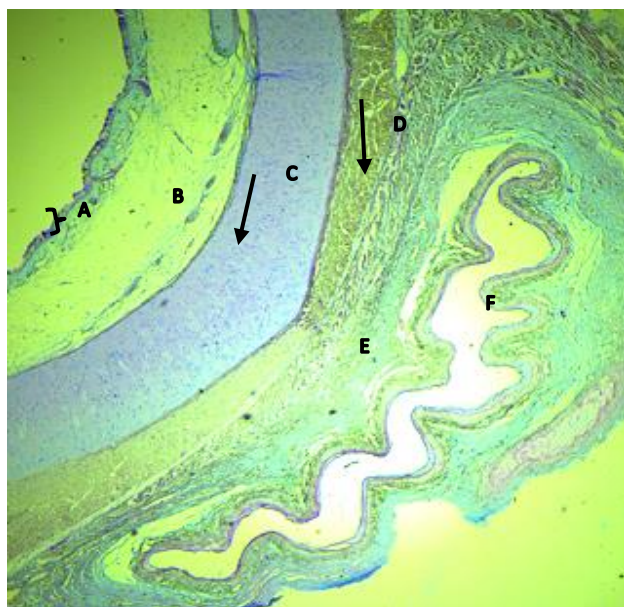


Fig.(2): Cross section of the cervical part of newly born lamb trachea; respiratory epithelium (A), goblet cells (B), blood vessels (C), submucosa (D), cartilage (E), chondrocyte (F), perichondrium (G), smooth muscle (H), PAS-AB, 100X

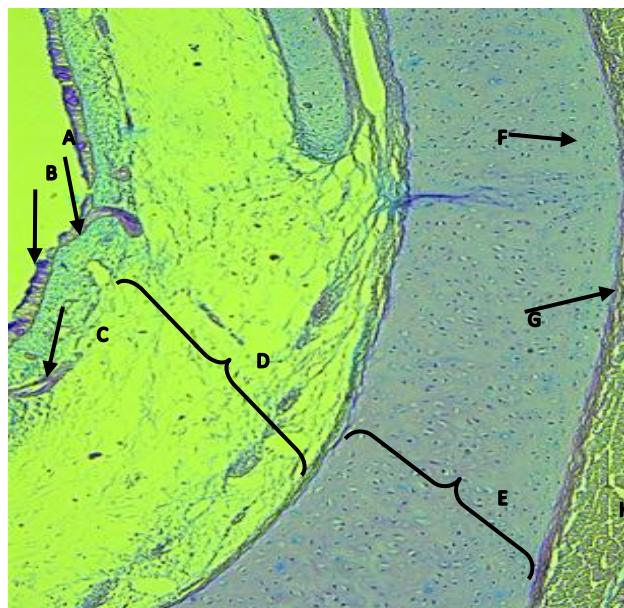


Fig.(3): Cross section of the thoracic part of newly born lamb trachea; respiratory epithelium (A), goblet cells (B), submucosa (C), blood vessels (D), cartilage (E), skeletal muscle (F), PAS, 100X

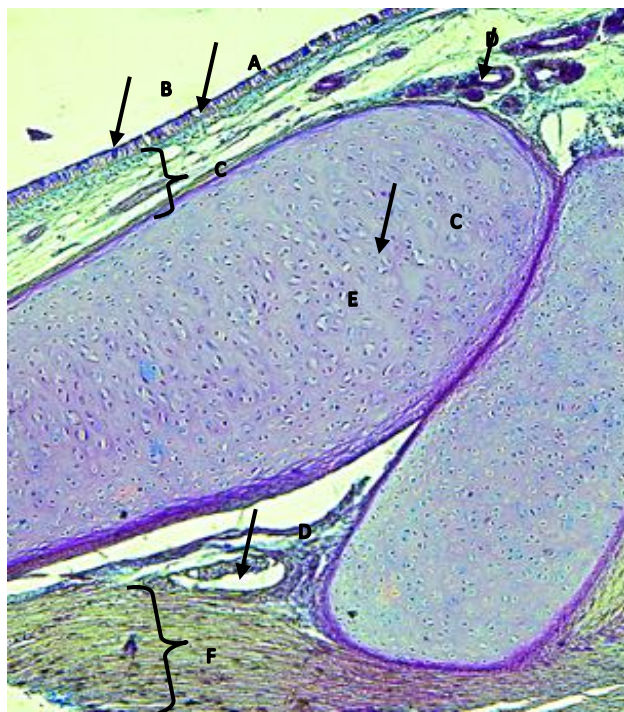


Fig.(4): Longitudinal section of the thoracic part of suckling lamb trachea; mucosa (A), ciliated cells (B), goblet cells (C), basal cells (D), submucosa (E), mucous glands (F), blood vessels (G), connective tissue (H), Masson, 200X.

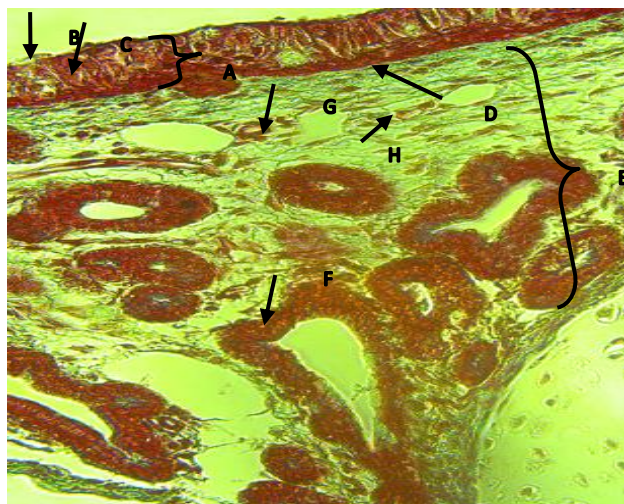


Fig.(5): Cross section of the cervical part of suckling lambs trachea; respiratory epithelium (A), goblet cells (B), submucosa (C), mucous gland (D), chondrocytes (E), perichondrium (F), Masson stain, 200X

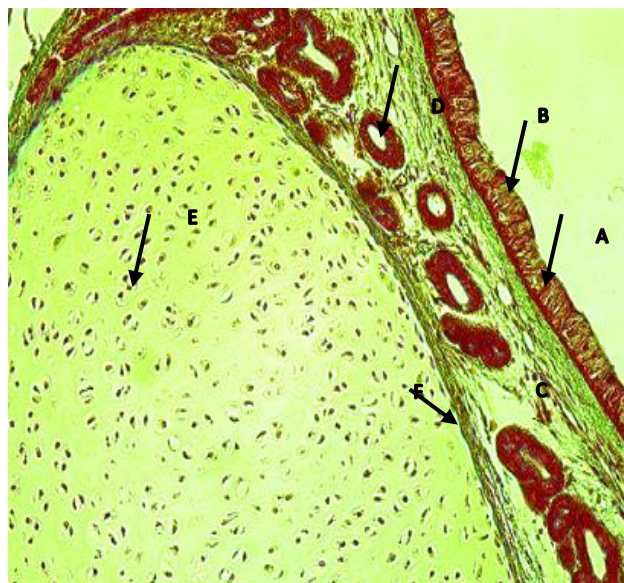


Fig.(6): Cross section of the cervical part of suckling lambs trachea; mucosa (A), glands in submucosa (B), cartilage (C), smooth muscle (D), adventitia (E), lumen of esophagus (F), Masson stain, 100X



Fig.(7): Cross section of the cervical part of newborn lamb trachea; respiratory epithelium (A), submucosa (B), cartilage (C), smooth muscle (D), chondrocytes (E), blood vessels (E), PAS, 200X

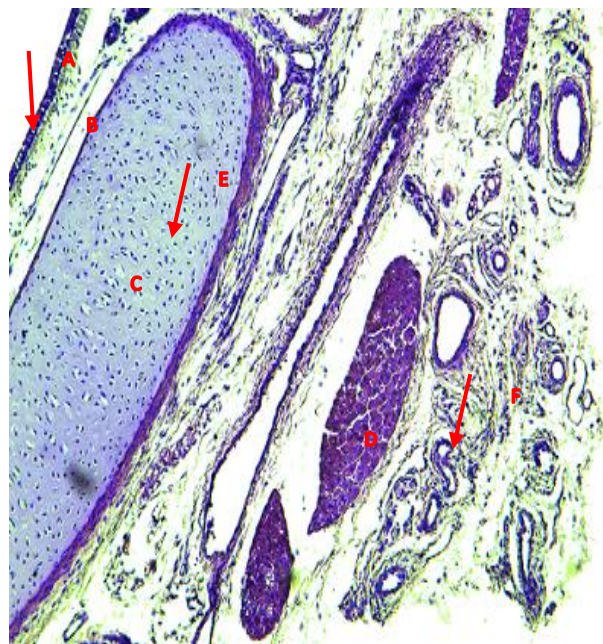
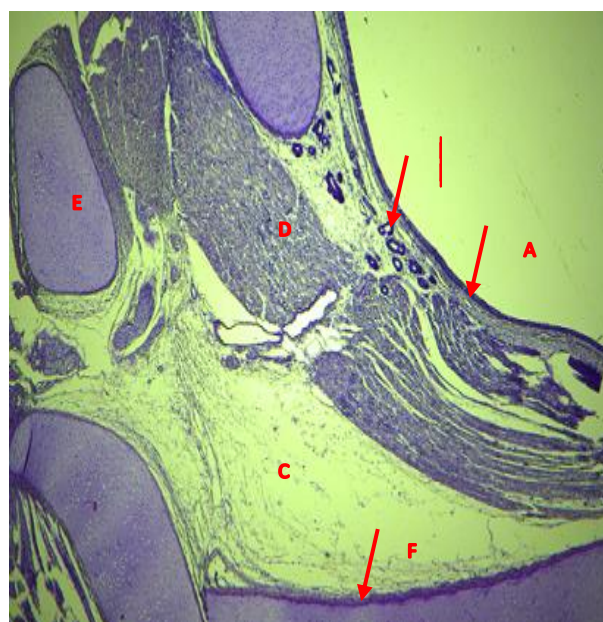


Fig.(8): Cross section of the thoracic part of suckling lamb trachea; respiratory epithelium (A), mucous gland (B), submucosa (C), smooth muscle (D), cartilage (E), perichondrium (F), PAS, 100X



Discussion

The trachea of rams has multiple layers; the mucosa is composed of a ciliated pseudostratified columnar epithelium and numerous goblet cells rest on a basement membrane, (17) gave similar description in domestic animals and similar respiratory epithelium and cells were described by (18) in camel (19) in Iraqi sheep and goat, and (20) in goats. In Camels, the tracheal mucosa characterized by the mucosal fold is short lined by respiratory epithelium containing four cell types ciliated, goblet intermediate and basal cells resting on the basement membrane (10,21).

In new born puppy dog the trachea has the folds in the dorsal wall cover by thick cilia (17). (20) who reported the trachea in goat is lined by the pseudostratified ciliated columnar epithelium with goblet and basal cells that rested on the basement membrane, of non ciliated cell, not described in rodent airway (6). The ciliated cells were completely in trachea of suckling lambs, non ciliated columnar epithelium in all trachea of newborn lambs, (1) noted that the tracheal ciliated cell of ovine and porcine an epithelial cells are specialized to transport secretion in the air way. These ciliated cell and are responsible for the transport of ions and water across the epithelia. The (3) noted that on the mammalian the tracheal epithelium cells types are thought to be secretory mucous cells, serous cells and non ciliated bronchiolar epithelial (Clara) cells. The goblet cells are also responsible for the formation of mucus spreading on the ciliated epithelial cells by the weather mean. The respiratory secretion mucus adherence with ciliated epithelial cells (1,2). Goblet cells, found throughout the length of the trachea Involved in the production of mucus, basal cells were wedged between the other

cells, as also described in domestic mammals (17).

The glands are mucous in newborn lamb trachea and seromucous coiled branched tubuloacinar in suckling lambs trachea, The goblet cells are present in signification number in the cat, sheep and monkey trachea (1). Our results indicated the presence of a large amount of both acidic and neutral polysaccharides in the trachea, The numbers of the goblet cells that have both acidic and neutral mucins were increased in the sucking age. These observations were in accordance with the findings made by (19) in sheep and goat

(2) in ox. These increased of goblet cells in this study to the protection. (3) who reported the most species in which the trachealis muscle attached, the internal surface of the cartilaginous ring, the gland were external to the muscle. In all species in which the muscle attached to the external surface of the cartilaginous rings, the glands were internal to muscle. In the goat, ox, dog and sheep, the glands in the ventral more than dorsal aspect of the trachea. Tracheobronchial glands are specialized extension of the air way surface epithelium into the submucosa (1). The (22) noted that tracheal gland in Giraffe, are composed of two types of the secretory cells; serous and mucous. Present internal to the mucosa. In camel the most submucosal glands are serous and few mucous (21). The tracheal glands were absent in Rabbit and guinea pig and in mouse (1). The muscle fibers are attached directly to the perichondrium, the attachment in some species (cat, dog, rabbit) to the external aspect of crescent of the trachea ring. In some guinea pig, hedgehog, man to their internal aspect, In rat the tip and both aspect of the crescents are attached directly (2). The cartilage matrix exhibited strong reaction

with PAS; chondrocytes, are showed weak reaction as in most mammals (1).

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