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Morphogenesis of the ostrich (*Struthio camelus*) trachea and lung in different embryonic and fetal stages

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Abstract

The ostrich (*Struthio camelus*) is an important wild species highlighted in national and international livestock industry. This research was conducted to analyze the development of the ostrich respiratory system during fetal and embryonic stages. A total of 50 fertile ostrich eggs were collected from commercial farms and then incubated at 36.00 - 37.00 ˚C and 25.00 ± 2.00% humidity for 40 days. Sections were taken on days 13, 22, 26, 30, 36, and 42 of incubation from the lung and the cranial, middle, and caudal parts of the neck after decapitation of ostrich embryos and blood drainage. After fixation, processing, blocking, and sectioning, all samples were stained by Hematoxylin and Eosin, Alcian Blue (AB), Van Gieson, and Periodic acid-Schiff (PAS) techniques. It was concluded that the trachea in the 13-day-old embryo and goblet cells (PAS-positive and AB-positive) had incomplete rings of hyaline cartilage and differentiation of mesenchymal to the loose connective tissue. The bronchial stage of the lung was observed in the 22-day-old embryo, pseudoglandular stage in the 26-day-old embryo, and parabrachial and air capillary stage in the 30-day-old embryo. The information obtained from this study will be useful for diagnosing pathologies affecting this vital system and results in improving industrial breeding management.

Introduction

The ostrich (*Struthio camelus*) is the largest and heaviest bird. It was known as the camel bird because of its prominent eyes, long neck, sweeping eyelashes, and high-temperature tolerance.

The anatomical structures play a major role in avian respiration, which differs fundamentally from respiration in mammals. In the *Paleopulmo,* there is a continuous caudocranial airflow during inspiration and expiration within the tertiary bronchi. The *Neopulmo*, found only in phylogenetically recent species, is characterized by tidal respiration.[1]

The respiratory system of birds plays a crucial role in the oxygen and carbon dioxide exchange. This system is also responsible for regulating heat, detoxifying metabolism, producing chemical messengers, and enabling vocalization.[2] Understanding the morphological aspects of embryonic and fetal development is important for breeding of ostriches on farms. However, there is still a lack of information regarding the morphology of the trachea and lung in ostriches during embryonic and fetal development.

The first morphological sign of respiratory tract development observed in chicken embryos (*Gallus gallus domesticus*) is the appearance of the laryngeotracheal groove. This groove becomes visible on the floor of the midline of the pharynx from the 3rd day of incubation, extending to the fourth pharyngeal arch. The groove closes along most of the length of the pharynx, forming a tube known as the trachea. It remains open to the pharynx at its forward end, forming the glottis.[3] The respiratory primordium is positioned and its territory is defined in the foregut. The visceral mesoderm of the respiratory primordium acquires the inducing potential necessary for endodermal budding morphogenesis and respiratory endoderm formation.[4] Several studies have been performed to describe the morphological characteristics of respiratory organs, primarily using domestic chicken (*G. gallus domesticus*) as an experimental model.[5-7] Other bird species such as ostrich (*S. camelus*),[8-10] parakeet (*Melopsittacus undulates*),[11] the quail (*Coturnix coturnix japonicum*),[12] the wild turkey (*Milagros gallopavo*),[13] and the pelican (*Pelecanus conspicillatus*),[14] [15] have also been used as models for studies in this area.

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This study aims to analyze the histological aspects of the respiratory system of the ostrich. The information obtained here will be useful for recognizing the ontogenetic pattern of the trachea and lung, as well as for other respiratory system investigations, and improving industrial breeding management.

Materials and Methods

A total of 50 fertile ostrich eggs were obtained from commercial farms. They were incubated at 36.00 - 37.00 ˚C and 25.00 ± 2.00% humidity. After decapitation of ostrich embryos and blood drainage, sections were taken on the 13th, 22nd, 26th, 30th, 36th, and 42nd days of incubation from the lung and the cranial, middle, and caudal parts of the neck. All samples were fixed in 10.00% buffered Bouin's solution (Merck Company, Darmstadt, Germany). After processing (dehydration with alcohol, clearing with xylene, and embedding with paraffin), blocks of paraffin were prepared using a paraffin dispenser (Didsabz, Urmia, Iran) and all blocks were sectioned with a thickness of 6.00 µm using a microtome (RM 2145; Leica, Wetzlar, Germany). The sections were mounted on slides and stained by Hematoxylin and Eosin, Alcian Blue (AB), Van Gieson, and periodic Acid-Schiff (PAS) techniques. All procedures were approved by the relevant Ethical Committee of Ferdowsi University of Mashhad, Mashhad, Iran (Code: IR.UM.REC.1402.156).

Results

The development of the trachea. In 13-day-old embryo (E13), the trachea appeared with a small lumen covered by undifferentiated stratified epithelium, surrounded by a dense layer of mesenchyme and tracheal cartilage was not yet observable by microscope (Olympus, Tokyo, Japan) at this stage (Fig. 1A, Table 1). Later on, this day, the epithelium gradually changed, and cilia began to spear.

In 22-day-old embryo (E22), the tracheal epithelium had developed into the pseudostratified columnar with the cilia and goblet cells (PAS-positive and AB-positive). Incomplete rings of hyaline cartilage and progression of mesenchymal differentiation into loose connective tissue were observed. Within the loose connective tissue of the lamina propria, small blood vessels and nerve endings were visible. In addition, intra-epithelial mucosal glands were observed in E22 (Fig. 1B, Table 1).

In 26-day-old embryo (E26), 30-day-old embryo (E30), 36-day-old embryo (E36), and 42-day-old embryo (E42), the trachea has continued to grow and develop. The epithelium consisted of ciliated pseudostratified columnar cells with goblet cells (Figs. 1C and 1D). Completed rings of hyaline cartilages, perichondrium, and well-developed intra-epithelial mucosal glands were observed in embryos E36 and E42 (Figs. 1E - 1F, Table 1).

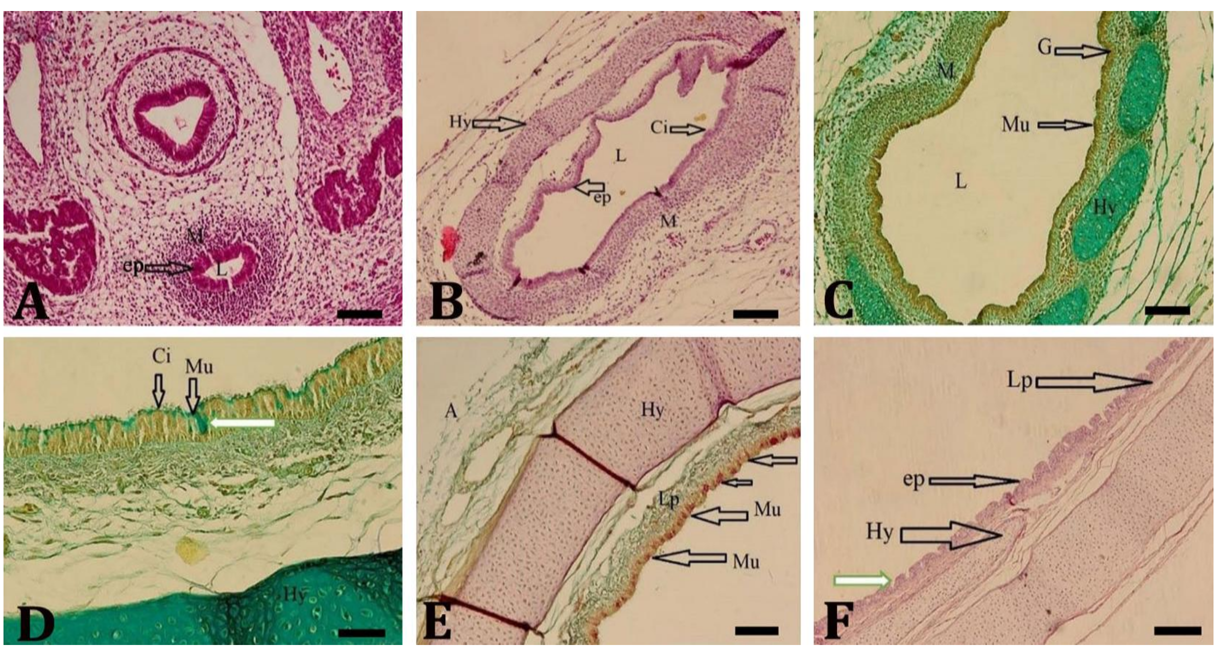

Fig. 1. Histological structures of the trachea on **A)** 13-day-old embryo, **B)** 22-day-old embryo, **C)** 26-day-old embryo, **D)** 30-day-old embryo, **E)** 36-day-old embryo and, **F)** 42-day-old embryo of the ostrich incubation period showed, ep: epithelium, L: lumen, M: mesenchymal cells, Ci: cilia, Hy: hyaline cartilage, G: intraepithelial gland. Mu: mucosa cell. LP: lamina propria. A: Adventitia layer, White arrows: Intra-epithelial gland (A, B, and F: Hematoxylin and Eosin; C and D: Alcian Blue/Van Gieson staining; and E: Periodic acid-Schiff staining, bars = 20.00 µm).

Table 1. Tracheal structure in several days of fetal ostrich.

Days	Epithelial types	Lamina propria	Cartilage	Cilia	Tissue types	Goblet cells	Mucosal glands
13	Stratified columnar	-	-	-	Mesenchyme	-	-
22	Pseudostratified columnar	+	+	+	Mesenchyme	+	+
26	Pseudostratified columnar	+	+	+	Mesenchyme	+	+
30	Pseudostratified columnar	+	+	+	Connective tissue	+	+
36	Pseudostratified columnar	+	+	+	Connective tissue	+	+
42	Pseudostratified columnar	+	+	+	Connective tissue	+	+

- : Absent, and +: Present.

The development of lung. Lung development was characterized by the stages of development of the ducts that form the intra-pulmonary bronchial system and the presence or absence of air and blood capillaries. These stages were bronchiolar, pseudoglandular, parabronchi, and air capillary. The bronchial stage was distinguished at E13 and E22 by the appearance of secondary and tertiary bronchi projecting into the mesenchyme. Additionally, pseudostratified epithelium with cilia, vascular connective tissue of lamina propria, longitudinal smooth muscle, and a submucosa layer were observed. The epithelium of the main bronchi was ciliated pseudostratified columnar with goblet cells (Fig. 2A). The pseudoglandular stage was characterized by a branch of the duct system (primary and secondary bronchi) resembling an endocrine gland; cells with globular and occasional blood vessels were observed in E26 of lung development (Fig. 2B). In the parabrachial and air capillary stages observed in E30, there was a high number of para-bronchi and increased vascularized lung tissue surrounding them. The main and secondary bronchi displayed epithelium consisting of a cuboidal basal layer and squamous superficial cells. Additionally, thick longitudinal smooth muscles were visible in the wall of the main bronchi (Figs. 2C - 2E). In the *S. camelus* at E42, similar lung structures were observed compared to those on days E30 and E36, with pneumocyte type I and II being more detectable in the alveoli on this day (Fig. 2F).

Also, the average thickness of different lung structures at various embryonic days in ostriches is shown in Table 2.

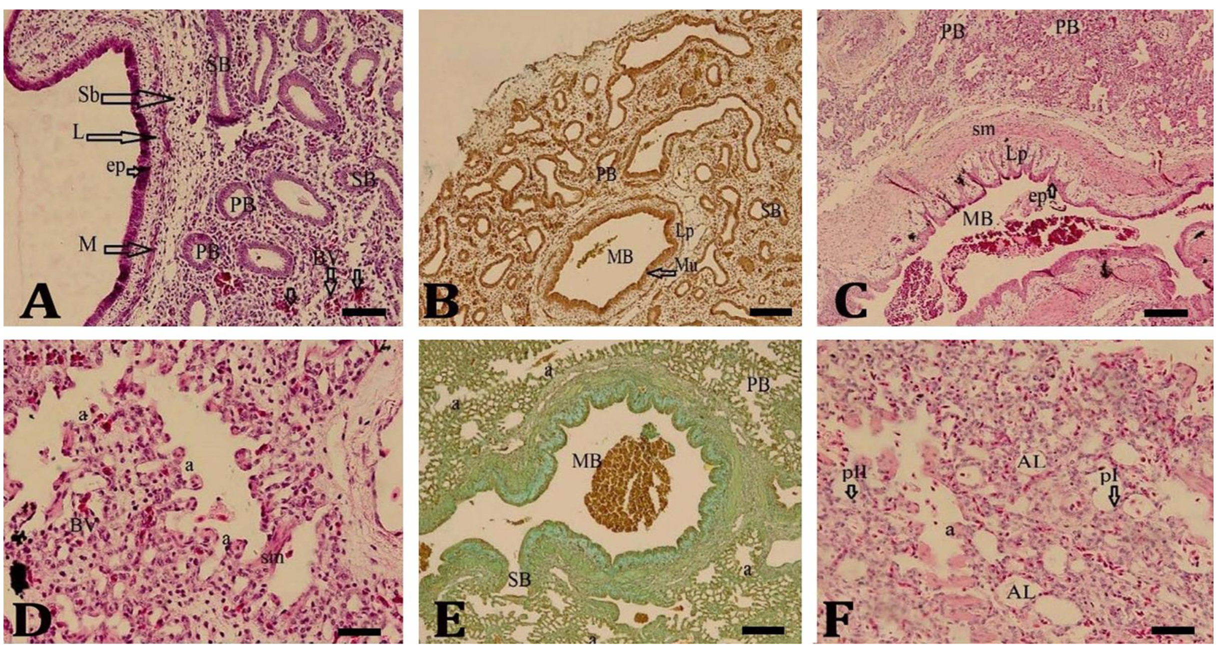

Fig. 2. Histological structures of the lung on, **A)** 13-day-old embryo, **B)** 22-day-old embryo, **C)** 22-day-old embryo, **D)** 30-day-old embryo, **E)** 36-day-old embryo and, **F)** 42-day-old embryo of the ostrich incubation period showed, SB: secondary bronchus, PB: parabronchus, M: muscle, ep: epithelium, L: lamina propria, Sb: submucosa, MB: main bronchi. Mu: mucosa cell. LP: lamina propria. Sm: smooth muscle, a: atrium, BV: blood vessel staining pI: pneumocyte I, pII: pneumocyte II. AL: alveolus (A, C, D, and F: Hematoxylin and Eosin; B: Van Gieson; E: Alcian Blue/Van Gieson staining, bars = 20.00 µm).

Table 2. The average thickness (µm) of different structures of the lung at various embryonic days in Ostrich.

Structures	Day 22	Day 26	Day 30	Day 36	Day 42
Secondary bronchi	326	252	523	600	418
Tertiary bronchi	61.40	56.10	92.20	77.40	122
Atrium	-	-	17.20	17.05	26.30
Air capillary	-	-	-	7.11	8.11

Discussion

Generally, the vertebrate lung originates from the endoderm in the region of the primitive foregut, where the epithelium gives rise to the airway system and the gas-exchanging units and the mesenchyme forms the connective tissue, muscles, and vessels. The lung starts as a primordium that splits into left and right buds each of which forms a respective lung. In birds, the lung buds form the primary bronchi from which the secondary bronchi arise. The parabronchi sprout from the secondary bronchi and occupy specific locations within the lung.[16] In avian embryos, in the early stages, cells form intraluminal protrusions (aposomes). Then, transcellular double membranes separate the aposome from the basal part of the cell establishing, unziping and severing the aposome from the cell. Additionally, better-endowed cells can squeeze out adjacent cells or such cells can constrict spontaneously thus extruding the squeezed out aposome.[17]

During the tracheal development of ostrich embryos and fetuses, the epithelium was stratified columnar without cilia and goblet cells. Furthermore, there were no lamina propria and cartilage present on E13 of the incubation period. By E22, the epithelium had developed into a pseudostratified columnar structure without cilia and goblet cells, but lamina propria and small cartilage particles had appeared. By E26 of the incubation period, all of the mentioned structures were fully recognizable.

The current study found that differentiation of epithelial cells in ostrich tracheal development occurs during the last stages of development, specifically between E26 and E30 of the incubation period. This is consistent with findings in domestic chickens, where differentiation occurs between day 10 and 21 of incubation.[18]

During normal morphogenesis of the tracheal epithelium, stem cells have the ability to differentiate into ciliated cells and mucus-secreting cells.[18] In this study, identification of ciliated cells was observed at E22.

Remodeling of intraepithelial glands at E30 of the incubation period has shown cellular organization similarities to what is observed in adult birds.[11,12]

A study of the development of tracheal cartilaginous rings has revealed that their formation started at E22 and was completed in E30 of the incubation period. Meanwhile, the trachea as a long, flexible cartilaginous tube is reported to be present at E6 in chickens and in the last days of the incubation period in rhea.[1] In the adult parakeet *M. undulates*, most tracheal cartilages have become calcified.[11]

The main extrapulmonary bronchi, also known as mesobronchi, were covered by typical respiratory epithelium as observed in ducks (*Anas platyrhynchos)*,[19] parakeets,[7] and domestic hens.[6]

The lungs of birds have a complex network of anastomoses that make them exceptionally efficient compared with those of mammals.[7] Five stages of development are traditionally identified in mammals' lung morphogenesis: embryonic, pseudoglandular, canalicular, saccular, and alveolar stages.[20] However, these developmental stages are not evident in the lungs of birds.[7]

We have identified specific structures of the pulmonary parabronchial phase in ostrich embryos between E26 and E30 of the incubation period, which were observed around day 10 in chickens.[6] In the later stages of ostrich development, the pulmonary parenchyma has shown a similar pattern to that of domestic chickens at day 21 of the incubation period.[21] At this stage, it is assumed that the atria are formed, and the blood-gas barrier (BGB) is developed, as identified after 18 and 32 days in domestic chickens and in pelicans (*P. conspicillatus*), respectively.[7] Other researchers studying the lung of ostrich embryos have found that between day 24 and 39, the epithelial thickness was reduced by 90.00 % from 13.50% ± 0.41 µm to 1.33 ± 0.01 µm and atria were evident at day 32, and the first portions of the BGB measuring 3.41 ± 1.12 µm were encountered at day 35.[22]

The morphogenetic study of the respiratory system in ostrich embryos contributes to identifying of the oncogenic pattern of this vital system in this species.

The information obtained in this research will be useful for identifying pathologies that affect the respiratory system, ultimately improving industrial breeding management. Further investigation is needed to assess the genetic and molecular factors involved in the morphogenesis of the respiratory system in ostriches.

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Conflict of interest

The authors declare no conflict of interest.

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