Prehatch Development of Respiratory System in Kuttanad Duck
(Anas Platyrhynchos Domesticus).

Firdous ahmad dar1., Maya Krishnan2, Ashok Narayan Pilli3, M. A. Baba4., Massarat Khan5
1,4,5 Division of Veterinary Anatomy and Histology, FVSc & AH, SKUAST-K
2,3Department of Veterinary Anatomy and Histology, CVAS, KV ASU, Kerala, India
Received: October 2015 ; Accepted: December 2015

ABSTRACT

The prehatch development of the respiratory system in Kuttanad ducks was studied by using 18 Kuttanad duck embryos collected on 7th, 14th and 21st day of incubation. In order to find out the respiratory primordia and its morphological development, few embryos were also collected from 3rd to 13th day of incubation. By 3rd day of incubation in the duck embryo, the olfactory pits were observed at the junction of the lateral and ventral walls of the head and was anterior to the primordia of eyes. By 7th day, the nasal cavity was formed and lined with a pseudostratified columnar epithelium. By 10th day of incubation the individual parts of the respiratory system were clearly observed with the stereozoom microscope. By day 14th day of incubation it was evident that the primary bronchi, secondary bronchi, parabronchi, air sacs and pleura came into existence. By 21st day the parabronchi developed and underwent micro-architectural organization with the cavitation that extended peripherally deep into the surrounding mesenchymal tissue all along the lung parenchyma. The atria and infundibulae were also developed. The development of the respiratory tract in prehatch period in the present study confirmed that the tract is an early maturing system in general and lung in particular.

Key words: Kuttanad, Prehatch, Primordia, Respiratory system

MATERIALS AND METHODS

The prehatch development of the respiratory system in Kuttanad ducks was studied by using 18 Kuttanad duck embryos collected on 7th, 14th and 21st day of incubation. In order to find out the respiratory primordia and its morphological development, a few embryos were also collected from 3rd to 13th day of incubation. The embryos were removed from the eggs at different ages and careful dissection was made in the embryos. Topography, position and relation of various components of respiratory system were noted by using a digital camera with 12x zoom (Nikon) and stereozoom microscope (Zeiss). The whole respiratory system was carefully removed and the morphometry including weight, length, breadth and thickness was recorded.

After recording the gross features, the materials were fixed 10 % neutral buffer formalin and Bouin’s fluid. After fixation in the appropriate fixatives, the materials were processed for paraffin embedding and sections of 5µm thickness were taken for histological and histochemical studies. The following staining techniques such as Haematoxylin and Eosin (H&E), Gomori’s rapid one step trichrome method, Ayoub Shaklar method, Safranin ‘O’, Periodic acid Schiff’s (PAS) and Alcian blue method for mucopolysaccharides , Best’s carmine method (Bancroft and Stevens, 1996) and ‘Oil Red’ O’ in propylene glycol method (Luna, 1968) were employed.

The morphometric and micrometric data were analysed statistically (Snedecor and Cochran, 1994). Pearson’s correlation coefficients (r) was used to find correlation if any.

RESULTS AND DISCUSSION

In the present study, the olfactory pits were observed at the junction of the lateral and ventral walls of the head and anterior to the primordia of eyes by 3rd day of incubation in the duck embryo.

*Corresponding author: drromey@gmail.com

34
The olfactory pits started to become deeper and migrated ventrally because of the rapid growth of forebrain above them. The mesodermal protuberances adjacent to nasal pits were the developing external and internal nasal processes. This was in accordance with the earlier reports of Romanoff (1960) in domestic fowl. The evaginated postbranchial region of the pharynx presented a midventral groove, the laryngo-tracheal groove or the primordium of the respiratory tract by this stage (Fig. 1). Romanoff (1960) also revealed this groove as the first indication of the formation of respiratory system in domestic fowl.

By 7th embryonic day, the nasal cavity was formed and lined with a pseudostratified columnar epithelium. The long laryngo-tracheal tube lay ventral and parallel to the esophageal primordium (Fig. 2). Street (1937) proposed that from the early developmental stages itself, the epithelium of the vestibule and the vestibular concha was nonsensory. The lungs attained a dorsal position and exhibited initiation of vascularization. By this age, near the base of the lungs lay the primordium of the extrapulmonary bronchus and formed the first branches of bronchial tree.

By 10th day of incubate the individual parts of the respiratory system, viz. nostrils, nasal cavity, nasal concha, pharynx, larynx, trachea and air sacs were clearly seen with the stereozoom microscope. By 14th embryonic day, the nostrils were apparently plugged by a mass of tissue, which prevented the communication of the nasal cavity to the exterior till hatch (Fig. 3). The respiration through nostrils was not necessarily needed during this period when, the chorioallantoic membrane (CAM) was acting as gas exchange medium between the exterior and the embryo (Bellairs and Osmond 1998). At the moment of hatch, this source of gas exchange will normally be vanished and the nostrils become patent by losing the nasal plug, which is of utmost importance for the survival of the duckling in the entirely new environment outside the egg.

In the present study all the three nasal conchae had attained their form by 14th day of incubation itself in Kuttanad ducks. This was in accordance with the reports of Michael (2004), who also revealed that even though the inferior concha was in a primitive stage in an eight-days-old chick, it was fully developed, attaining its final form by 14th day of incubation. These gross observations in the present study especially that of nasal conchae can be attributed to the better differentiation at cephalic end during embryonic period.

Histologically, in the 14th day embryo, the nostril mucosa exhibited an undifferentiated layer of cuboidal cells without any keratinization. Epidermis and dermis were not clearly demarcated (Fig. 4). Larynx presented the laryngeal opening and the trachea was a transparent cartilaginous tube on the ventral aspect of the developing neck by 14th days of incubation in the present study (Fig. 5). The extrapulmonary primary bronchi were also visible at this stage as two thin transparent tubes. These observations partly confirmed those of Tymms (1913), who reported the differentiation of trachea-bronchial rings and their supporting framework by 12th day.

Syrinx was in the form of a slightly dilated organ with its supporting framework at the base of the heart in 14-days-old Kuttanad duck embryos (Fig. 6). These observations partly agreed with those of Romanoff (1960) who observed the muscles of the syrinx developing by 15th day of incubation in chick and also with those of Alcantara et al. (2013), who stated that the respiratory organs in chicken were completely differentiated by 10 days of incubation. By day 14th day of incubation, grossly the lungs were greyish-white in colour and came to occupy its dorsal wall of the thorax, indented by ribs dorsally. Microscopically, it was evident that the primary bronchi, secondary bronchi, parabronchi, air sacs and pleura came into existence (Fig. 7). Most of the parabronchi were canalised and presented a lumen but still, some of the parabronchi were represented by aggregations of epithelial cells without canalisation, indicating the continuing differentiation and development (Fig. 8).

By 21st day of incubation, nostrils which were still plugged, but histologically presented four layers, viz. stratum basale, stratum spinosum, stratum granulosum and stratum corneum in epidermis (Fig. 9). All the three conchae became clearly visible and the nasal cavity was lined by three different types of epithelium. Olfactory epithelium appeared like an elongated paint brush and bone olfactory hair at the apical border. Development of the four layers of the epidermis at very early stage and the presence of three different types of epithelium lining the nasal cavity suggested that the protective role of the skin came into existence when the duck embryo was still inside the egg. Development of early olfactory epithelium in the Kuttanad ducks might be helpful in the postnatal life for differentiating the food odours during foraging.

By 21st day of incubation, the developing respiratory system was well set with all the three conchae clearly visible, larynx with laryngeal opening carrying sagittal row of papillae on its sides and the firm and wedge-shaped lungs with six shallow indentations made by ribs. The mesenchymal tissue was almost negligible in the lung by this age. The parabronchi developed and underwent micro-architectural organization with the cavitation that extended peripherally deep into the surrounding mesenchymal tissue all along the lung parenchyma. The atria and infundibulae were also developed.

The development of the respiratory tract in prehatch period in the present study confirmed that the tract is an early maturing system in general and lung in particular. This indicated that the lung-air sac system of birds at hatching was practically mature as against the mammalian lung where intensive growth of the terminal airways and important structural changes occur postnataally (Adamson, 1997).

Mean body weight of Kuttanad ducks at different age groups, mean total weight of respiratory tract and its percentage contribution to body weight was given in table 1. The mean total weight of respiratory tract from larynx to lungs by 14th and 21st days of incubation was 0.121 ± 0.001 and 0.622 ± 0.031g respectively and
showed a significant positive correlation with age and bodyweight (r=0.939 and r=0.936). The mean weight of the lungs at 14th and 21st days of incubation was 0.320 ± 0.001 and 0.620 ± 0.001 g respectively. The mean total weight of respiratory tract of Kuttanad duck embryos during the days of incubation showed a significant positive correlation with the age, indicating a progressive development to adapt with a totally different environment inside the egg.

**REFERENCES**


**Table 1.** Mean body weight, weight of respiratory tract and its percentage contribution to body weight during prehatch period in Kuttanad ducks (Mean±S.E)

<table>
<thead>
<tr>
<th>Incubation period (days)</th>
<th>Body weight (g)</th>
<th>Weight of respiratory system (g)</th>
<th>Percentage contribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>7th</td>
<td>0.550±0.003</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>14th</td>
<td>4.075±0.001</td>
<td>0.121 ± 0.001</td>
<td>2.96</td>
</tr>
<tr>
<td>21st</td>
<td>17.400±0.004</td>
<td>0.622±0.031</td>
<td>3.57</td>
</tr>
<tr>
<td>28th</td>
<td>35.600±1.32</td>
<td>1.820±0.003</td>
<td>5.11</td>
</tr>
</tbody>
</table>

**I.L.S. of 3-day old kuttanad duck embryo. H&E x400.**

**2. L.S. of 7th day old kuttanad duck embryo. H&E x400.**

**3. Head of 14th day old kuttanad duck embryo.**
1. Blocked nostril, 2. Upper beak, 3. Eye

**4. C.S. of 14th day old kuttanad duck embryo. H&E x400.**
1. Undifferentiated epidermis, 2. Dermis

**5. Larynx and trachea of 14th day old kuttanad duck embryo.**
1. Larynx, 2. Trachea, 3. Extra pulmonary primary bronchus

**6. Syrinx of 14th day old kuttanad duck embryo.**
1. Syrinx, 2. Extra pulmonary primary bronchus, 3. Lung, 4. Trachea
7. Cross section of chest cavity of 14th day old kuttanad duck embryo


9. 21st day of incubation, nostrils