

SURFACE MORPHOLOGY OF THE DEVELOPING RESPIRATORY EPITHELIUM OF CHICKS

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SUMMARY

The development of the respiratory tract of chicks between the age of 15-day-old embryos until 3-day-old chicks was carried out using scanning electron microscopy (SEM). The distribution of ciliated and microvillous cells was investigated and given a score of 1 to 4 throughout the entire surface of the respiratory tract which include the middle nasal concha, larynx, cranial trachea, caudal trachea, intrapulmonary primary bronchus and secondary bronchus. The surface morphology of the intrapulmonary primary bronchus and secondary bronchus attained a mature densely ciliated epithelium as early as in the 15-day-old embryos. However, in the 19-to 20-day-old embryos, this was only found in the epithelium of the middle nasal concha, larynx, cranial and caudal trachea.

Keywords: surface morphology, respiratory epithelium, chicks

INTRODUCTION

The respiratory epithelium of chicks plays an important role in the defense of the respiratory tract against irritants and infectious agents (Breeze and Wheeldon, 1977; Toth and Siegel, 1986; Toth *et al.*, 1987). The state of development of the respiratory epithelial lining at hatching is therefore of prime importance, since respiratory-related diseases have been shown to be the principal cause of chick mortality, especially within the first three weeks of life (Hofstad, 1984; Morris, 1992; Alexander, 1994; Bekker *et al.*, 1995; Jones, 1996), and are of significant economic importance within the poultry industry (Leong *et al.*, 1994; Cook, 1996). Moreover, in recent years there has been an increase in the use of scanning and transmission electron microscopy of the normal and pathological respiratory epithelium in the chicken. Most of the information available, however, concentrates on the adult respiratory epithelium, with very little information apparently available on the development of the respiratory epithelium of the incubating chick. Such studies as are available appear limited to either selected regions of the respiratory tract, such as the middle nasal concha (Breipohl and Fernandez, 1977), larynx and trachea (Kalnins and Porter, 1969; Kalnins *et al.*, 1972; Walsh and McLelland, 1974a; b; c; 1978; Mohamed, 1989) or lung (Jones and Radnor, 1972a; b; Duncker, 1978). It is the purpose of the present study to provide for the first time, an ultrastructural study of the development of the respiratory epithelial lining of the entire tract of the incubating chicks from the 15-day-old embryo through to the 3-day-old post-hatched chick.

MATERIALS AND METHODS

Sixty fertilized eggs from breeder hens which had not been exposed to formaldehyde were obtained from a

commercial supplier and incubated at 37.5 °C (Brinsea Multihatch incubator). Six embryos per day were randomly selected on days 15, 16, 17, 18, 19 and 20 of incubation, day-old and 3-day-old chicks. All chicks were sacrificed and preserved accordingly for scanning electron microscopy. Each chick was euthanised using an overdose intracardiac injection of sodium pentobarbitone (Euthatal; May and Baker). The respiratory tract was removed and the middle nasal concha, larynx, cranial trachea, caudal trachea, intrapulmonary primary bronchus, secondary bronchus and tertiary bronchus were collected from preselected sites. These samples were fixed in 4 % buffered glutaraldehyde and processed and later analysed using scanning electron microscope (EMSCOPE SC 500). The progress of the development was judged according to the features below using scanning electron microscope while the transmission electron microscopic results were used to support the features seen under SEM.

Characterisation of the developing respiratory epithelium

- 1+ - predominantly microvillous cells, with or without cilia
- 2+ - patches of developing ciliated cells
- 3+ - extensive patches of mature or fully developed ciliated cells
- 4+ - predominantly covered with dense fully mature ciliated cells.

RESULTS

The distribution and nature of the cells lining the developing respiratory epithelium are summarised in Table 1. In middle nasal concha, larynx, cranial trachea and caudal trachea of the 15, 16, 17 and 18-day-old embryos, the respiratory epithelium was either lined with predominantly microvillous cells, with or without cilia (Fig. 1), patches of developing ciliated cells (Fig. 2) or extensive patches of



Fig. 1. Middle nasal concha, 15-day-old embryo. Note the typical cobblestone appearance of the mucosal surface, and well demarcated borders(—) of the individual microvillus cells both raised and flattened. Pits on the microvillus cell (arrow) and occasional appearance of microplike (open arrow). X 5,500

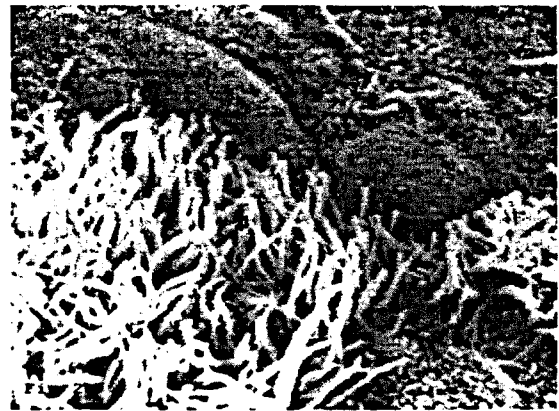


Fig. 2. Middle nasal concha, 17-day-old embryo. All three stages of developing cells can be seen in this scanning electron micrograph. Note: 1. Non-ciliated microvillus cell with single cilium. 2. Early stage; microvillus cell with single cilium. 3. Immature stage; numerous cilia are appearing. 4. Mature stage; fully developed ciliated cells. X5,500



Fig. 3. Middle nasal concha 3-day-old chick. Epithelial surface with mucous granules (arrow) at the opening of the intraepithelial gland opening (open arrow). X5,500

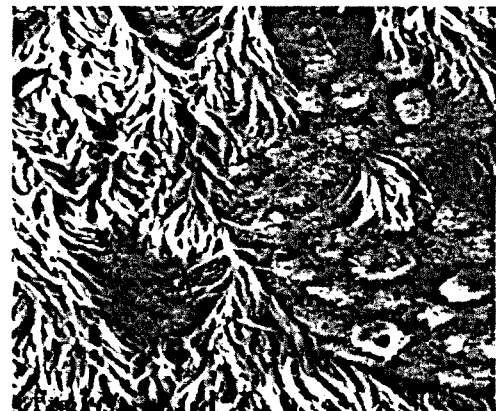


Fig. 4. Caudal trachea 3-day-old chick. Dense carpet of cilia interrupted by islands of microvillus cells on the mucosal surface. Note: pits (arrow) and numerous mucous granules (open arrow) X2,750

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Table 1: Distribution of ciliated and microvillous cells in selected regions of the respiratory tract of the developing chick

Regions	Day-old embryos					Day-old chicks		
	15	16	17	18	19	20	1	3
Middle nasal concha	1+	2+	2+	3+	4+	4+	4+	4+
Larynx	1+	1+	2+	3+	3+	4+	4+	4+
Cranial trachea	1+	2+	2+	3+	3+	4+	4+	4+
Caudal trachea	1+	2+	2+	3+	3+	4+	4+	4+
Lungs: Intrapulmonary primary bronchus	4+	4+	4+	4+	4+	4+	4+	4+
Secondary bronchus	3+	3+	4+	4+	4+	4+	4+	4+

mature or fully developed ciliated cells (Fig. 3). The dense fully mature ciliated cells were predominantly seen on the surface of these regions in the 19 and 20-day-old embryos as well as in the 1 and 3-day-old chicks. However, in the lungs these mature ciliated cells coverage could be seen in the intrapulmonary primary bronchus as early as in the 15-day-old embryo while in the secondary bronchus, it was first seen in the 17-day-old embryo. Mature mucous cells were noted characterised by sparsely distributed microvilli on their bulging surface, which also demonstrated numerous surface pits and mucous granules (Fig. 4). This feature could be observed throughout the respiratory tract of embryos and chicks in all the age groups. As for the tertiary bronchus it could not be summarised according to the criteria stated earlier. The tertiary bronchus was lined by non-ciliated microvillous cells exhibiting cobblestone appearance in 15 and 16-day-old embryo. The atria were not obvious since the parabronchial lining cells were compacted together at this stage. In the older embryos and chicks (17-day-old embryos to 3-day-old chicks), the atria became clearly recognisable and surfactant was present on the mucosal surface.

DISCUSSION

The present study demonstrated that developing ciliated cells could be distinguished from the surrounding stubby microvillous cells by the presence of a single cilium, as seen in the proximal regions of the respiratory tract in the 15-to 17-day-old embryos. The surface appearance of just such a single cilium has also been documented in the respiratory tract of rabbit foetus (Kanda and Hilding, 1968) and in the nasal mucosa of man (Friedman and Bird, 1971).

The observation, that a fully differentiated, mature, heavily ciliated epithelial lining, similar to that of the adult chicken (Mohamed, 1989), is established in the intrapulmonary primary bronchus of the developing avian embryo by as early as the 15th day of incubation. The secondary bronchus is covered by extensive patches of ciliated cells even in the 17-day-old embryo, whilst the middle nasal concha, larynx and trachea do not become fully ciliated until later, between 19 to 20 days of incubation. Such a regional variation in ciliary development, is

described here for the first time in the incubating chick embryo, with ciliogenesis progressing along a caudo-rostral axis, contrasts with previous reports of developmental ciliogenesis in mammals, where cilia first appear in the nasal cavity of the rabbit at 22 days of gestation, followed by the appearance in the larynx, trachea and finally the bronchi at 28 days of gestation (Kanda and Hildings, 1968). Such ciliary development has also been described in the dog (Wright *et al.*, 1983), where it was additionally noted that the tracheal and bronchial epithelial linings were not completely ciliated until the puppy was 5 days old. Again, the observation contrasts markedly with the present finding that the entire respiratory epithelium was covered with densely packed fully mature, ciliated cells as early as the 19th day of incubation. The present study demonstrates that a fully developed respiratory epithelium is established throughout the tract by the time the chick hatches which are in agreement with earlier SEM studies of the epithelial surface of the middle nasal concha of chicks (Breipohl and Fernandez, 1977). Although intracellular mucous granules were seen, the actual mucus secretion onto the epithelial surface was not noted until the 18th day of incubation, and was most active in the 3-day-old chick. It is tempting to speculate that the initiation and increase in mucous cell activity started with the beginning of air breathing as a result of internal pipping by the chick. Such an event, which precludes hatching, occurring between the 18th to 20th day of incubation (Duncker, 1978; Burton and Tullet, 1985), coinciding with the full development of a mature ciliary carpet lining the respiratory tract. The presence of mature ciliated cells and mature mucous cells comprising the major cell populations of the epithelial lining of the respiratory tract of the developing chick embryo from the 18th day onwards, indicate that the chick hatches with a fully functional mucociliary system in place as an integral component of the respiratory defense mechanisms. The presence of intraepithelial mucous gland openings throughout the respiratory tract, contradicts the findings of Chandra and Bharadwaj (1971), that mucous glands of the chicken were restricted to regions proximal to the cranial trachea. However, intraepithelial mucous glands have been described in the secondary bronchus of the budgerigar (Smith *et al.*, 1987).

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RINGKASAN*MORFOLOGI PERMUKAAN PEMBENTUKAN EPITELIUM PERNAFASAN ANAK AYAM*

Perkembangan pembentukan saluran pernafasan anak ayam berumur dari 15 hari embrio sehingga 3 hari anak ayam telah dijalankan menggunakan mikroskop elektron pembias (SEM). Taburan sel silia dan sel mikrovilus telah dikaji dan diberi skor 1 ke 4 ke atas keseluruhan permukaan saluran pernafasan termasuk konka nasal tengah, larinks, trakea kranial, bronkus primer intrapulmonari dan bronkus sekunder. Morfologi permukaan bronkus primer intrapulmonari dan bronkus sekunder memperoleh epitelium bersilia yang lebat seawal dalam anak ayam berumur 15 hari embrio. Walaubagaimanapun, dalam embrio berumur 19 ke 20 hari, hanya dapat dilihat pada permukaan epitelium konka nasal tengah, larinks dan trakea kranial.