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Structural Development, Cellular Differentiation and Proliferation of the Respiratory Epithelium in the Bovine Fetal Lung

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Summary

Fetal bovine lung samples of 11 different gestational ages were assigned to a classical developmental stage based on histological morphology. Immunohistochemistry was used to characterize the morphology of forming airways, proliferation rate of airway epithelium and the presence of epithelial cell types (i.e. ciliated cells, club cells, neuroepithelial cells (NECs) and type II pneumocytes). Typical structural organization of pseudoglandular (84–98 days gestational age [DGA]), canalicular (154–168 DGA) and alveolar (224–266 DGA) stages was recognized. In addition, transitional pseudoglandular–canalicular (112–126 DGA) and canalicular–saccular (182 DGA) morphologies were present. The embryonic stage was not observed. A significantly ($P < 0.05$) higher proliferation rate of pulmonary epithelium, on average 5.5% and 4.4% in bronchi and bronchioles, respectively, was present in the transitional pseudoglandular–canalicular phase (112–126 DGA) compared with all other phases, while from 8 weeks before term (224–266 DGA) proliferation had almost ceased. The first epithelial cells identified by specific marker proteins in the earliest samples available for study (84 DGA) were ciliated cells and NECs. Club cells were present initially at 112 DGA and type II pneumocytes at 224 DGA. At the latest time points (224–226 DGA) these latter cell types were still present at a much lower percentage compared with adult cattle. This study characterized bovine fetal lung development by histological morphology and cellular composition of the respiratory epithelium and suggests that the apparent structural anatomical maturity of the bovine lung at term is not matched by functional maturity of the respiratory epithelium.

Keywords: cattle; development; lung; respiratory epithelium

Introduction

The structure of mammalian lungs is highly species dependent (Plopper *et al.*, 1983; Warburton *et al.*, 2000) as are the timings and patterns of epithelial cell differentiation during gestation and postnatal development (Plopper *et al.*, 1980a,b; Hyde *et al.*, 1983; Plopper *et al.*, 1992a; Plopper and Fanucchi, 2004). Mammalian lungs undergo five recognized morphological developmental phases from conception to parturition: embryonic, pseudoglandular, canalicular, saccular and alveolar (Burri, 1997; Corrin, 2000). In the earliest embryonic phase, the rudimentary trachea and lungs differentiate from the endoderm of the foregut and the main conducting airways develop from the first clusters of epithelial cells. The branching of these conducting airways corresponds to the branching of the initial pulmonary blood vessels and the main

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lobulation of the pulmonary mesenchyme also develops at this stage. The pseudoglandular phase, named because of the morphological resemblance of airway ducts in cross-section to glandular tissue, is characterized by intensive formation of additional conducting airways, which become bronchi with associated cartilage and bronchioles. By the end of this phase terminal bronchioles are present. The canalicular phase is characterized by narrowing of the distal ends of the terminal bronchioles and the appearance of the first airspaces which will, in the subsequent saccular phase, develop by the process of septation into sacculi (pocket-like structural units). During the saccular phase blood vessels develop a close proximity to tissue destined to undertake gaseous exchange. In the final alveolar phase, the sacculi transform by secondary septation into millions of alveoli, which are the primary units of gaseous exchange (Burri, 2006; Morrisey and Hogan, 2010). Generally, rodents are born with their lungs at the saccular phase, while people are born with their lungs at the alveolar phase (Pinkerton and Joad, 2000). In man, the alveolar phase first appears approximately 4 weeks before birth (36 of 40 weeks gestational age [WGA]) and alveolarization continues postnatally for up to 2 years (Schittny and Burri, 2008). In cattle, a single report based on the examination of 60 bovine fetuses described the relationship between age of gestation, derived from measuring the crown–rump length and other morphological features, and lung developmental phases by histological morphology (de Zabala and Weinman, 1984). The study estimated that the alveolar phase began by 34 WGA, which is at least 6 weeks prior to parturition (based on a gestation period of 280–290 days for cattle). In sheep, gestation is shorter (148 days), but the alveolar phase of lung development is present 4 weeks before birth (Alcorn *et al*., 1981). Therefore, for both of these ruminant species the alveolar phase appears at approximately 80% of the gestation period while in man the alveolar phase does not appear until 90% of the gestation period has passed.

Concurrent with the development of conducting airways and alveoli during gestation, the respiratory epithelium differentiates into a number of highly specialized cells, with a variety of functions, which enable gaseous exchange and also protect the lung from invading pathogens and noxious agents (Knight and Holgate, 2003). The main epithelial cells present within conducting airways are ciliated epithelial cells, goblet cells, club cells (formally known as Clara cells [Winkelmann and Noack, 2010]) and basal cells (Corrin, 2000). The majority of epithelial cells found within the gaseous exchange region are type I and type II pneumocytes (Corrin, 2000). In addition, single neuroepithelial cells (NECs) and clusters of several NECs, named neuroepithelial bodies (NEBs), are present in both the conductive and respiratory areas of lung, but in low numbers (Van Lommel, 2001).

As the rate and timing of appearance of the various respiratory epithelial cells during gestation has not been reported for cattle, the aims of this study were to relate, in bovine fetuses of varying gestational age, histological lung morphology and detailed composition of respiratory epithelium in terms of the number and distribution of the principal cell types and their proliferative activity.

Materials and Methods

Tissue Samples and Processing

A total of 15 fetuses from clinically healthy cows, used as negative control animals from experiments conducted previously (Maley *et al*., 2003; Macaldowie *et al*., 2004; Benavides *et al*., 2012), were selected. As determined by insemination dates, a sample from the left caudal lung lobe was collected from two fetuses each of 84, 98, 112 and 126 days gestational age (DGA) and from one fetus of 154, 168, 182, 224, 238, 252 and 266 DGA. The samples were fixed in 10% neutral buffered formalin and processed routinely prior to embedding in paraffin wax. Sections (4 μm) were stained with haematoxylin and eosin (HE).

Determination of Phase of Lung Development

Under blinded conditions each lung sample was assessed by histological morphology and assigned, where possible, to one of the five phases of development recognised in human lungs (i.e. embryonic, pseudoglandular, canalicular, saccular and alveolar) (Corrin, 2000) or to a transitional phase between two adjacent developmental phases when the morphology present was not typical of any single phase. The lung phase was then aligned, retrospectively, with the day of gestational age.

Immunohistochemistry

An anti-pan-cytokeratin antibody was used to label all of the respiratory epithelial cells in order to visualize developing airways. Ciliated respiratory epithelial cells were labelled by targeting the β-tubulin protein club cells by club cell secretory protein (CCSP), type II pneumocytes by surfactant protein-C (SPC) and dendritic cell-lysosomal associated membrane protein (DC-LAMP), NECs by the