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Development of Respiratory Tract from Bovine Embryos

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We studied the development of respiratory tract in bovine embryos by light microscopy and transmission electronic microscopy. This process was observed formation of laryngeal-tracheal tube in embryos that present crown-rump (CR) length of 9.0 mm (20/21 days of pregnancy) at around the fourth gestational week; the organ wall appears to be formed of epithelium comprising several cell layers and supported by mesenchyme. Within the lungs, the areas subjacent to epithelium present condensed mesenchyme, while more distal areas loose mesenchyme, in which blood vessels beginn to form, as the organ is in a pseudo-glandular phase. Ultra-structurally, the mesenchyme cells present irregular shapes, having a stellar or fusiform appearance and are united by desmosomes, where the cytoskeleton attaches to the cellular membrane, forming a connection. The bifurcation of trachea caudal portion in the main bronchia is simultaneous to the appearance of the tracheal bronchi, during the fifth gestational week.

Key words: Lungs, tracheal bronchi, electron microscopy, respiratory system, embryology

INTRODUCTION

The development of inferior respiratory tract organs in human has been described by Duplessis (1970); Pringle (1986); Larsen (1997); Moore et al. (2002); Moore and Persaud (2004); and Sadler (2005), who also suggested that the origin of the respiratory primordium appears approximately at embryonic day 28 as a median sulcus at the caudal end of the primitive pharynx ventral wall: the laryngeal-tracheal crevice. At the end of the fourth week, the crevice invaginates to form a sac-like respiratory diverticulum, located in the ventral region of the caudal portion of the anterior intestine, when the stomodeum and primitive intestine fuse and membranes disintegrate (Crelin, 1976). The pulmonary buds to branch off and contain the surrounding mesenchyme, followed by mesenchymal expansion that forms segmental lobules (Hislop, 2002).

Peters et al. (1994) and Cardoso (1995) state that the mouse lungs originate from an epithelial bud of primitive intestine endoderm, in which epithelial tubules are surrounded by loose mesodermal cells that appear in each compartment for differentiation. The early stage extends the proliferation of these tubes, generating a ramifying system that soon establishes the mature organ model; these ramifications and distinct phenotypic cells appear along epithelial tubules associating with mesenchymal components of lungs development.

The developing human lungs are lined by mesothelial lamina that protrude to the sides in their respective pleural cavities. The mesenchyme that involves the bronchial tree gives rise to tissues (cartilages, smooth muscle, conjunctive tissues) surrounding the bronchia epithelial wall (Lobo, 1966).

O'Rahilly (1984) showed that in human embryos at gestational day 26, the oral-pharyngeal membrane vanishes and pulmonary bud development occurs; at day 28, pulmonary bud separation from the digestive tube occurs with a reorganization of esophagus and trachea. At day 32, pulmonary sacs form dorsal bowl-like shapes and surround the esophagus, followed by a connection of the pharyngeal-tracheal duct to digestive and respiratory tubes, and by lobar bud development. At day 37, epithelial lamina completes itself and separates the digestive from respiratory tube, initiating primary palate formation. At day 41, the development of segmental bronchial buds begins, and at day 44, some subsegmental buds appear.

Garcia et al. (1991) and Sadler (2005) complement this work, showing that in human the respiratory diverticulum separates from the primitive pharynx through the tracheal-esophageal septum derived of tracheal-esophageal crests, which differentiate a completely respiratory tissue from the digestive portion.

Simultaneously to pulmonary organogenesis, development of vascular connections occurs (Burri, 1984). Until gestational day 28, groups of endothelial cells are fused around the mesenchyme in the ventral diverticulum. At day 34, the capillary plexus surrounds the two pulmonary buds, and is connected to pulmonary arteries and veins (Hislop, 2002).

According to Hyttel et al. (2010) in domestic animals the development of the bronchi and lungs can be divided into sequential periods: the embryonic period, when the primor-

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dium of the bronchi and lungs are formed; the fetal period, when the ramified bronchi are formed and the preliminary structures for the gas exchange are established; and the postnatal period, when the definitive structures for gas exchange, the alveoli, are developed and the lungs assume their adult form. The fetal period can be subdivided into overlapping pseudo-glandular, canalicular, saccular, and alveolar periods.

Despite the great knowledge acquired in past years, especially in the last century, current scientific literature provides a limited description of bovine embryology. Due to ease of study, much has been studied about the embryology of humans and small animals, while morphological studies in large animals have been to some extent neglected. The aim of the present study is to provide microscopic descriptions of the development of respiratory tract in bovine embryos between 20 and 46 days of development, in order to contribute to the scientific community increasing studies in this species.

MATERIALS AND METHODS

Collections of uteri from half-breed cows were performed in cold storage depots and slaughterhouses in the Dracena-SP and Poços de Caldas-MG region. We obtained a sample of 50 embryos at different gestational stages. Our studies were conducted according to ethical guidelines approved by the Regional Scientific-Ethical Committee on the Use of Animals at the School of Veterinary Medicine of São Paulo, University of São Paulo, Brazil (protocol n° 846/2006).

The uteri were opened and the embryos fixed in 2.5% Bouin and glutaraldehyde solution. The embryos were transported to the Laboratories of Anatomy and Histology, Medical College of Zoötechny and Veterinary Medicine/FMVZ – USP. The gestational periods were estimated following the methodology approved by Winters et al. (1942); Evans and Sack (1973); Noden and Lahunta (1990) with measurements of occipital-sacral distance (Crow/Rump/CR) (Brydens et al., 1972; Kaufman and Bard, 1999).

In the light microscopy processing, the embryos were put in Bouin fixing solution, in which they were immersed by 24 hours until their complete fixation. After this processing, the material was dehydrated in a series of increasing concentrations of ethanol (from 50 to 100%) and diaphonized in xylol, followed by embedding in paraplast® (Parablast Embedding Media_Paraplast Plus, Sigma, Oxford, Lab., USA). This was performed using consecutive cuts of 5-μm of thickness, stained by routine methods (hematoxilin-eosin) for posterior description and documentation using photomicrography.

In the transmission electronic microscopy, the embryos were previously fixed in 2.5% glutaraldehyde (glutaraldehyde grade I: 70% aqueous solution – Sigma Chemical Co., USA) in 0.1 M phosphate buffer, pH 7.2. At the end of fixation, the material was washed in 0.1 M sodium phosphate buffer, pH 7.4, and post-fixated in 1% osmium tetraoxide (4% osmium tetra-oxide w/w solution in water – Polysciences, Inc., USA). After new washes, the embryos were dehydrated in ethyl alcohol at 50%, 70%, 90%, and 100% and washed in propylene oxide (propylene oxide EM Grade, Polysciences, Inc., USA).

For 16 hours, the embryo fragments stayed under rotation at 1:1 propylene oxide and resin (Araldite – 502 Embedding, Kit Electron Microscopy Sciences, USA). In sequence, the fragments were embedded in moulds of pure resin, remaining in an oven at 69°C for 72 hours. Semi-fine cuts of 1-μM of thickness were obtained, and hot stained with a 1% sodium borate solution in distilled water, containing 0.25% Toluidine Blue. Ultra-fine cuts of about 60 nm of thickness were collected in copper screens and contrasted with 2% uranil acetate and 0.5% lead citrate.

RESULTS

We observed laryngeal-tracheal tube formation in embryos with CR of 9.0 mm (20/21 days of pregnancy). Epithelium, which was supported in mesenchyme, constituted of several cells layers formed the laryngeal-tracheal tube wall. Moreover, we noted the presence of a tracheal-esophageal pleat, delimiting the laryngeal-tracheal tube from the esophagus, being that both to find themselves connected to the embryo dorsal region through of a mesenchyme lamina: the dorsal mesentery (Fig. 1).

The tracheal bronchus, a characteristic specific to bovine species, was observed in embryos with CR lengths of 12.0 mm (28/30 days of pregnancy). By consecutive sectioning, we could note the presence of trachea and tracheal bronchus, followed by its bifurcation, giving rise to the main bronchia and persistence of tracheal bronchus. In embryos with a CR of 17.0 mm (32/34 days of pregnancy), total separation of laryngeal-tracheal tube from esophagus occurred, and in the median plane ventral to trachea, we observed the main bronchial buds (Fig. 1).

Each bronchus was constituted by epithelium presenting several cells layers, supported in mesenchyme. Delimiting the epithelium and mesenchyme area, we noted the presence of the basal membrane. Several cells layers presenting an irregular shape, grouped and united with each other, with an absence of extracellular material, composed the epithe-

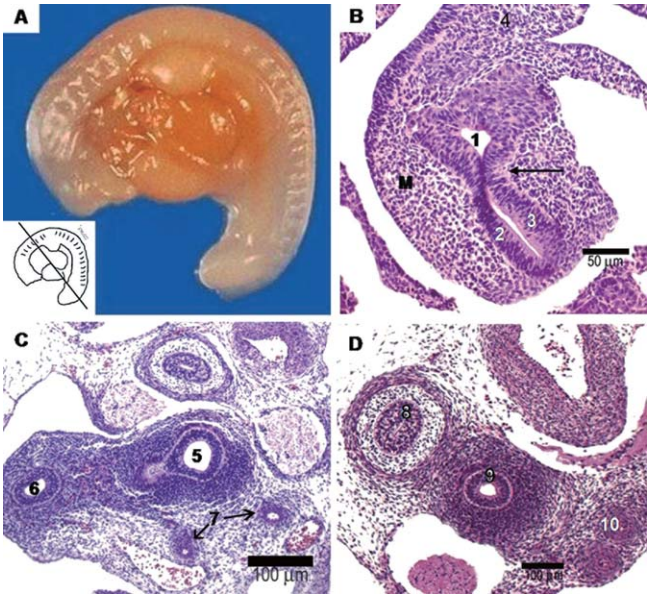

Fig. 1. **(A)** Photograph of bovine embryo in lateral view showed crow-rump (CR) length = 9.0 mm (20/21 days of pregnancy); **(B)** Photomicrograph of bovine embryo with CR = 9 mm (20/21 days of pregnancy) showing tracheal tube with esophageal portion (1) and tracheal portion (2), epithelium (3), dorsal mesentery (4) and mesenchyme (M) and trachea-esophageal fold (→); **(C)** Protomicrographs of bovine embryo with CR = 12 mm (28/30 days of pregnancy) showing tracheal tube (5), tracheal bronchus (6) main bronchus (7); **(D)** Photomicrograph of bovine embryo with CR = 12 mm (28/30 days of pregnancy) observing early formation of the main bronchi, esophagus (8) positioned dorsally to the trachea (9) and ventrally at the main bronchi (10).

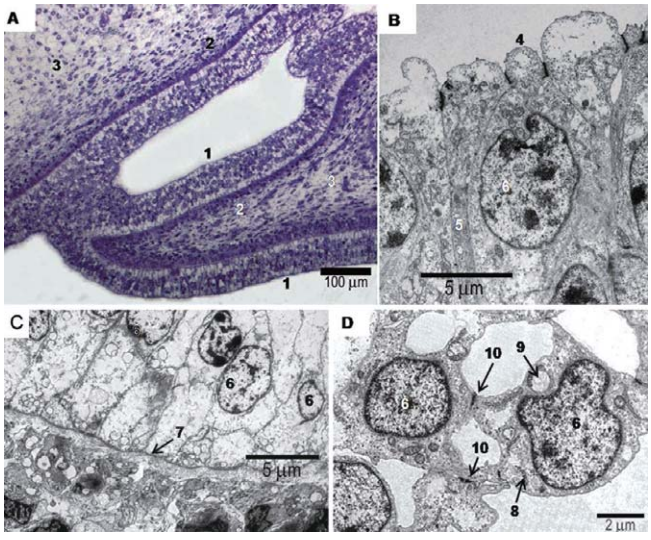

Fig. 2. **(A)** Photomicrograph of bovine embryo showed the segmental bronchus in formation with crown-rump (CR) length = 17.0 mm. (32/34 days of pregnancy). Lung epithelium (1) consisting of disorganized cell layers, the area of condensed mesenchyme (2) around the bronchus and loose mesenchyme (3) in more remote regions. **(B)**, **(C)** and **(D)** Electron micrographs of bovine embryo with CR = 17.0 mm. (32/34 days of pregnancy) of epithelial cells from segmental bronchus bovine embryo. **(B)** and **(C)** the apical pole of epithelial cells can be noticed the presence of blisters (4), membranes (5), nucleus (6), the basal membrane (7) delimiting epithelial and mesenchymal area. In **(D)**, the nucleus (6) have more rounded shapes, rough endoplasmic reticulum (8) and vacuole (9) in the cytoplasm and the presence of desmosomes (10) promoting the approach of cells between two adjacent epithelia.

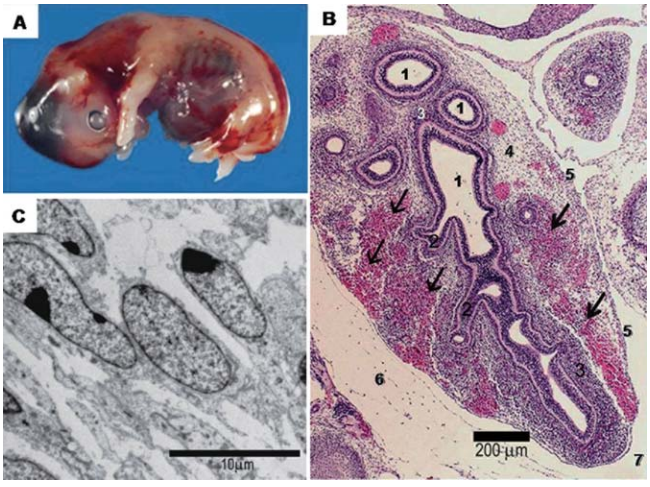

Fig. 3. **(A)** Photograph of bovine embryo in lateral view with crown-rump (CR) length = 27.0 mm. (45/46 days of pregnancy). **(B)** photomicrograph of bovine embryo lung phase pseudoglandular watching the main bronchi (1), bronchial side (2), an area of intense vascularization (arrows), condensed mesenchyme (3), loose mesenchyme (4), visceral pleura (5), the parietal pleura (6) and pleural cavity (7). **(C)** electron micrographs showing condensed mesenchyme cells proximal to each other with small area of extracellular matrix.

lium. The epithelial cell nucleus was globular or fusiform, reflecting the cell shape,; the nucleolus was present and chromatin was homogeneously distributed forming granules. At apical pole of epithelial cells, we observed plasmatic membrane projections and vesicles constituted by membrane (Fig. 2).

In the area subjacent to epithelium, we visualized condensed mesenchyme while in more distal regions mesenchyme was looser, where blood vessels were present. Ultra-structurally, the mesenchyme cells present a bulky nucleus and nucleolus, showing chromatin regularly distributed forming granules of variable size dispersed in nucleoplasm and chromatin adhered to nuclear covering. In the cytoplasm of these cells, we verified the presence of rugous endoplasmic reticulum, and in some cells identified vacuoles (Fig. 2).

Fetuses with CR of 27.0 mm (45/46 days of pregnancy) presented lungs in pseudo-glandular phase, with subdivisions of pulmonary lobules in cranial and caudal, together with their ramification originating of a main bronchus. The organ presents a large amount of blood vessels (Fig. 3).

DISCUSSION

The formation of laryngeal-tracheal tube appears in bovine embryos at a CR length of 9.0 mm (20/21 days of pregnancy), at around the fourth gestational week, from an epithelial bud of primitive intestine endoderm. The wall of this tube is formed by epithelium constituted of several cells layers and supported in mesenchyme. When compared with descriptions of Duplessis (1970); Crelin (1976); Moore et al. (2002); Moore and Persaud (2004), we confirmed that the development of lungs accompanies the appearance and development of bronchial arcs.

The early stage in humans extends proliferation of these tubes, generating a ramification system, which soon establishes the mature organ model (Peters et al., 1994; Cardoso, 1995). We verified that in bovines this coincides with the appearance of the tracheal bronchus, accompanying this ramification system initiated by division of trachea caudal portion in the main bronchia, but is independent, its bifurcation departing cranially from trachea, and according Dyce et al. (2004), in post-natal period, its function is to ventilate the right cranial lobe.

We observed that occurs the bifurcation of tracheal caudal portion in the main bronchia, simultaneously to t4racheal bronchi appearing, in embryos with CR of 12.0 mm (28/30 days of pregnancy) at around the fifth gestational week. From this process, the division of main bronchia begins in secondary (lobar) and segmental bronchioles. O'Rahilly (1984) states that in humans, segmental bud development begins at gestational day 41, and that of sub-segmental buds at day 44.

Each lobar bronchus is composed of epithelium presenting several cell layers, and supported in mesenchyme; in the area subjacent to epithelium, condensed mesenchyme is present and in more distal areas mesenchyme is looser. It was believed that condensed mesenchyme is responsible for bronchial cartilage and smooth muscle origin, while loose mesenchyme is the source of conjunctive tissue, responsible for the shape and sustentation of the organ. Simultaneously to pulmonary organgenesis, it occurs vascular connections development (Burri, 1984). With a CR of 17.0 mm (32/34

days of pregnancy), fifth gestational week, the capillary plexus surrounds the two pulmonary buds, being in connection to pulmonary arteries and veins (Hislop, 2002).

In bovine species, in the beginning of fetal period with CR of 27.0 mm (45/46 days of pregnancy), the lungs, still in the pseudo-glandular phase, present a large amount of blood vessels that are beginning vascularization, even before it passes to the canalicular stage, distinct from in humans in which formation of lung begins at 28 gestational days, as described by Crelin (1976); Duplessis (1970); Moore et al. (2002); Moore and Persaud (2004).

The mesoderm cells tend to differentiate, associating mesenchyme components to pulmonary development (Peters et al., 1994; Cardoso, 1995). In our studies, the mesenchyme cells have presented irregular shapes, "united" with each other at specific structures named desmosomes, where cytoskeleton to become attached to cellular membrane, forming a connection. This information led us to understand that intense intercellular communication is required to generate the inductive factors responsible for organ development. Wessells (1970) and Bluemink et al. (1976) also reported an extra-cellular material constituted by an amorphous interstitial substance and collagen fibers, which, in sequence, differentiates itself in collagen.

We observed a basal membrane that delimits the mesenchymal area from the epithelial. The epithelial tissue is composed of several cell layers with an irregular shape, grouped and united with each other, with a small amount or absence of extracellular material. The nucleus shape reflected the cell shape, with chromatin distributed in a homogeneous manner and only a single nucleolus, distinct from the report by Bluemink et al. (1976), which states that the nucleus can contain one or more nucleoli. Perhaps the number of nucleoli increases with posterior cellular differentiation.

We conclude that observation of the development of the respiratory tract in bovine embryos reveals that the formation of the laryngo-tracheal tube appears in bovine embryos at 20/21 days of development from an epithelial bud from the endoderm of the primitive intestine; the bifurcation of the caudal portion of the trachea in the main bronchi is simultaneous to the appearance of the tracheal bronchus in embryos with gestational age of 28 days; and the division of the cranial-caudal lung lobes begins in embryos at 44 days of development. We emphasize the need for further studies investigating the development of the respiratory tract at specific gestational ages and different periods from those analyzed in this study, in order to enable a full comparative study in bovine embryology.

REFERENCES

Bluemink JG, Maurik PV, Lawson KA (1976) Intimate cell contacts at the epithelial/mesenchymal interface in embryonic mouse lung. J Ultrastruct Res 55: 257–270

Burri PH (1984) Fetal and Postnatal development of the lung. Ann Rev Physiol 46: 617–628

Bryden MM, Evans HE, Binns W (1972) Embryology of the sheep. J Morphol 138: 169–186

Cardoso WV (1995) Transcription factors and pattern formation in the developing lung. Am J Physiol 269: L429–L442

Crelin ES (1976) Development of the upper respiratory system. Clin Symp 28: 3–30

Duplessis H (1970) Embriologia: Cuadernos Practicos, Barcelona: Ed Barcelona, p 144

Dyce KM, Sack WO, Wesing CJG (2004) Tratado de Anatomia Veterinária. Editora Elsevier, Rio de Janeiro, p 813

Evans HE, Sack WO (1973) Prenatal development of domestic and laboratory mammals: Growth curves, external features and selected references. Zentralbl Veterinarmed C 2: 11–45

Fonseca JF, Silva Filho JM, Pinto Neto A, Palhares MS (2001) Estágio de desenvolvimento embrionário de vacas zebuínas superovuladas. Arq Bras Med Vet Zootec 53: 6

Garcia SM, Jeckel Neto LE, Fernandez CG (1991) Embriologia Humana. Porto Alegre: Artes Médicas, p 350

Hislop AA (2002) Airway and blood vessel interaction during lung development. J Anat 201: 325–334

Hyttel P, Sinowatz F, Vejlsted M (2010) Essentials of Domestic Animals Embryology. Elsevier Health Science, Oxford, p 472

Kaufman MH, Bard JBL (1999) The Anatomical Basis of Mouse Development. USA: Academic Press, p 291

Lobo BA (1966) Noções Básicas de Embriologia Humana. Guanabara Koogan, Rio de Janeiro, p 318

Moore KLT, Persaud VN (2004) Embriologia Clínica. Guanabara Koogan, Rio de Janeiro, p 609

Moore KLT, Persaud VN, Shiota K (2002) Atlas Colorido de Embriologia Clínica. Guanabara Koogan, Rio de Janeiro, p 284

Noden DM, Lahunta A (1990) Embriología de Los Animales Domésticos. Acribia, Barcelona, p 399

O'Rahilly R (1984) Respiratory and alimentary relations in staged human embryos. Ann Otol Rhinol Laryngol 93: 421–429

Peters K, Wener S, Liao X, Wert S, Whitsett J, Williams L (1994) Targeted expression of a dominant negative FGF receptor blocks branching morphogenesis and epithelial differentiation of the mouse lung. EMBO J 13: 3296–3301

Pringle KC (1986) Human fetal lung development and related animal models. Clin Obstet Gynicol 29: 502–513

Sadler TW (2005) Jan Langman – Embriologia Médica. Guanabara Koogan, Rio de Janeiro, p 347

Wessells NK (1970) Mammalian lung development: interactions in formation and morphogenesis of tracheal buds. J Exp Zool 175: 455–466

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