Canine pulmonary surfactant synthesis during late fetal development
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Introduction
Histological lung maturation comprises five phases of development: embryonary, pseudoglandular, canalicular, saccular and alveolar. The canalicular phase is characterized by the development of the type I and type II pneumocytes, responsible for surfactant synthesis. The major function of surfactant SP-B protein is to reduce alveolar surface tension and thus, prevent lung collapse at the end of the expiratory phase. Impairment in SP-B production renders the newborn unsuitable for extra-uterine respiration. Therefore, the extent of pulmonary surfactant production can certainly be correlated with fetal maturity. This study aimed to determine the gestational period from which the onset of surfactant SP-B production begins in canine fetuses.

Material and methods
A total of 13 fetuses of different breeds were utilized. Animals were allocated into three groups, according to gestational period: 45 to 49 days of gestation (n = five), 50 to 54 days of gestation (n = five) and 55 to 63 days of gestation (n = three). After fetal euthanasia, fetuses were submitted to pulmonary lobectomy. The fetal lungs were washed plentifully with saline solution and then, samples were removed from each lobe. Lung lobes were cut into 2 cm³ fragments and stored in 10% formalin at room temperature until processing. The lung fragments were embedded in paraffin, cut into 5 µm cross sections and subjected to immunohistochemistry reaction with primary specific antibody for surfactant protein SP-B (Chemicon International, Temecula, CA) and a biotinylated secondary antibody (DakoCytomation, Carpinteria, CA). The sections were counterstained and evaluated under optical microscopy (10-100X) for a positive immunostaining at pneumocytes type II cells.

Results and discussion
Surfactant SP-B protein was identified in pneumocytes type II cytoplasms from all fetuses with at least 50 days of gestational age. In canine fetuses, the canalicular phase of lung development corresponds to the interval between the 48th and 57th days of pregnancy. Thus, it is possible to infer that the initial SP-B expression is similar among dogs and humans. However, respiratory system maturation depends on a successful process of morphogenesis and angiogenesis, adequate surfactant production and composition and proper fetal adaptation to stress. Our results will allow for the accurate indication of hormonal therapy for the stimulation of fetal maturation. Since previous studies concerning fetal lung development and surfactant production in canine species are limited and scarce, future investigations are needed to broaden our understanding in this area.

Keywords: Surfactant; SP-B protein; immunohistochemistry; lung development, dog

Reference