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Elastographic and echotextural characteristics of foetal lungs and liver during the final 5 days of intrauterine development in dogs

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ABSTRACT

Objective was to evaluate the echotexture and characteristics during terminal development of canine foetal respiratory and hepatic systems through elastographic examinations. Fifteen pregnant bitches were evaluated by ultrasonography twice daily, from the 53rd gestational day until whelping, and images obtained from 120 to 0 h before parturition were analysed. Images of foetal lungs and liver were recorded and then used for computer-assisted analyses to determine quantitative attributes. Acoustic Radiation Force Impulse (ARFI) elastographic of internal organs were classified as 'soft' (white areas) or 'hard' (dark areas) and quantitative analyses determined the mean shear wave velocities (SWV) of foetal lungs and liver. After delivery, canine neonates were clinically evaluated, and their health status was monitored weekly until 60 days post-partum. Sonographic parameters over time were compared by ANOVA and Pearson's correlations were used to determine associations between SWVs and echotextural variables. Foetal lungs and liver had a homogeneous echotexture and pulmonary parenchyma appeared hyperechoic when compared with that of the liver. Mean numerical pixel values (NPVs) of lungs decreased from 120 to 24 h and subsequently increased until parturition ($P = 0.04$). Lungs and liver mean (± SD) SWVs (0.98 ± 0.12 and 0.84 ± 0.11 m/s, respectively) didn't vary ($P > 0.05$) over time. Fluctuations in pulmonary NPVs indicated there was a pattern corresponding to structural and functional changes that occur during the terminal stage of pre-natal canine development and hence can be a useful diagnostic tool in veterinary. Foetal lung and liver SWVs were relatively consistent and there was no detectable changes during the pre-partum period for this variable or in echotexture.

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1. Introduction

Prematurely born neonates frequently suffer from developmental disorders of the pulmonary system associated with relatively greater mortality rates (Rades et al., 2004) owing to respiratory distress syndrome (RDS; Martin and Fanaroff, 2013). When there is unassisted birth of puppies with RDS, there is a reported mortality rate of 25% and 25% of live puppies develop bronchopulmonary dysplasia (Cornfield, 2013).

Silva et al., (2015) reported the L/S (lecithin to sphingomyelin) ratio in the amniotic fluid and found that this ratio correlates with lung maturity and neonatal health status, with a low ratio indicating compromised respiratory function and requiring intensive care in the immediate neonatal period. In general, the underdevelopment of either pulmonary or hepatic tissues leads to a functional impairment in respiration and metabolism (Martin and Fanaroff, 2013).

The histological development of dog lungs has been amply described (Sipriani et al., 2009) and was divided into the pseudoglandular (between 35th-48th days of gestation), canalicular (from gestational days 49–56), saccular (from 57th-60th day of gestation), and alveolar phases, occurring during early post-natal life. During the canalicular phase, the appearance of pneumocytes begin the final stage of the development and maturation, culminating in surfactant production. The RDS is caused by the underproduction of surfactant due to the immaturity of type II pneumocytes, which results in inadequate function of the immature alveoli.

Pulmonary ultrasonography has the potential as a viable and non-invasive technique, for estimating the maturation of foetal human lungs (Beck et al., 2015; Serizawa and Maeda, 2010). Campbell (1969) validated the foetal parietal diameter as an indirect indicator of lung maturity and ultrasonographic evaluation of lung echo in humans and comparing it to the hepatic echotexture has yielded highly promising results for the clinical monitoring of foetal lung development and the prediction of the onset of neonatal RDS (Serizawa and Maeda, 2010; Beck et al., 2015). Shah and Graham (1986) determined that ultrasonographically-estimated placentation grade was correlated with lung maturity, and Podobnik et al. (1996) suggested that the echotextural attributes of lungs correlated with foetal maturity. Lastly, Kim et al. (2013), using spectral Doppler sonography, determined the blood flow indices of the foetal pulmonary artery and found that some of these were indicative of the development of RDS in the neonates.

The imaging methods that have been developed specifically to evaluate the elasticity of tissues are collectively referred to as elastography and are classified, based on the source of the force that is exerted on the tissues, as acoustic radiation force impulse (ARFI) and real-time shear velocity (RSV). In addition, elastographic evaluations can be performed using a qualitative technique known as strain elastography and a quantitative technique utilising compression waves (Feliciano et al., 2015c). In veterinary medicine, the ARFI ultrasonographic technique has been used to detect various pathological changes in the canine and spleen and kidneys of cats as well as in the liver, prostate gland, and testes of dogs (Holdsworth et al., 2014; Feliciano et al., 2015a, b; Garcia et al., 2015; Maronezi et al., 2015). This technique also promises to be a valid method of assessing structural changes in foetal lungs and liver that may be indicative of the maturation; previous studies by Quarello et al. (2016) in baboons and by Zheng et al. (2016) in humans defined the normal ranges for foetal lung shear wave velocity during gestational development. There, however, have been no earlier systematic studies in humans or animal species of veterinary interest using foetal pulmonary and hepatic elastography during the prenatal period.

Based on these findings, the primary aim of the present study was to determine the echotextural and elastographic characteristics, and reference ranges for shear velocities of canine foetal lungs and liver in the last 5 days of gestation. Ultrasonographic image attributes (B-mode) were also determined for correlations between shear velocities (ARFI) of foetal pulmonary and hepatic tissues. It was hypothesised that both non-invasive techniques would provide useful information on the terminal development of foetal lungs and liver in dogs.

2. Materials and methods

All experimental procedures were approved by the Animal Ethic and Welfare Committee (Univ Estadual Paulista) protocol N°11765/14. Fifteen clinically healthy, primiparous or multiparous bitches of different breeds (four Dobermann Pinschers, seven Shih-tzus, and four French Bulldogs) owned by commercial dog breeders (body weight 7.0 ± 3.6 kg and age 2.5 ± 1.2 years) were used in this study.

After oestrous confirmation (vaginal cytology), all animals underwent intrauterine artificial insemination (AI) with fresh semen every 24 h for three consecutive days (Jacomini et al., 2006).

Ultrasonographic pregnancy detection (Acuson S2000™ ultrasonic device; Siemens®, Munich, Germany equipped with a 9.0 MHz linear transducer) was performed 2 weeks after the first AI as in a previous study by Feliciano et al. (2007). All ultrasonographic examinations were performed by one experienced (5 years) operator to reduce the evaluation time and stress endured by pregnant animals, and to consistently ascertain the proper development of conceptuses and their gestational age (Socha et al., 2012; Yeager et al., 1992).

Because the length of gestation in dogs is variable (between 57 and 63 days; Concannon et al., 1983), subsequent ultrasonographic evaluations of foetal lungs and liver were performed twice daily, from 53 days after the first AI (canalicular phase) until whelping (saccular phase of pulmonary development). For pregnancies with greater than or equal to three foetuses, only three different foetuses were evaluated, and in the other animals all foetuses were examined. To evaluate the same three foetuses in each pregnant bitch that had more than three conceptuses, only the two caudal foetuses (one from each uterine horn) and a foetus in the uterine body region were evaluated.

The transducer was positioned in the caudal abdominal region and all adjustable settings of the ultrasonic device (e.g., depth, gain, mechanical index and focal zones) were optimised and then left unchanged for the entire study period. Ultrasonograms