Theriogenology 96 (2017) 158–163

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

# Estimation of fetal lung development using quantitative analysis of ultrasonographic images in normal canine pregnancy

T. Banzato, DVM, PhD *, G. Zovi, DVM, C. Milani, DVM, PhD

*Department of Animal Medicine, Production and Health, Clinical Section, Radiology Unit, University of Padua, Viale dell'Università 16, AGRIPOLIS, Legnaro 35020, Padua, Italy*

A R T I C L E I N F O

*Article history:*
Received 2 December 2016
Received in revised form
6 March 2017
Accepted 14 March 2017
Available online 18 March 2017

*Keywords:*
Pregnancy
Echogenicity
Lung
Liver
Fetal maturity

A B S T R A C T

We investigated the quantitative analysis of sonographic images to predict fetal lung maturity of the canine foetus in normal pregnancy. Twelve bitches were recruited in the present study. Serial ultrasonographic exams were performed at three pre-determined time periods corresponding to the pseudoglandular (40–48 days of pregnancy), canalicular (49–56 days of pregnancy) and saccular phase (57–63 days of pregnancy) of lung development. Mean grey level (MGL) and the standard deviation of the histogram (SDH) of fetal lung and liver sonographic images were measured with dedicated software. The lung-to-liver ratio (LLR) for both parameters was also calculated. Measurements were taken on the two caudal-most foetuses and then averaged. SDH did not show any statistically significant difference between the three time periods in the lungs or in the liver. MGL measured in the lungs significantly increased in the first period and reached a plateau during the last two periods. Liver echogenicity was constant during the first two periods and significantly increased during the last week of gestation. The LLR of MGL significantly decreased during the last week of pregnancy. The LLR was a very good test to detect fetal lung maturity (area under the receiver operator curve (AUROC) = 0.875); using a cut-off value of LLR < 1.541, sensitivity was 83.33% and specificity was 83.33%, positive likelihood ratio = 5. LLR of MGL is an accurate test to estimate lung development in normal canine pregnancies.



## 1. Introduction

The physiologic range of pregnancy in canines varies widely when calculated from multiple (57–72days) [1] or single matings (58–69 days), both due to variable duration of behavioural oestrus (3–21 days), and prolonged sperm viability in the female reproductive tract [1]. This variability is important to be considered for selecting the day in which to perform a cesarian section in order to minimize pregnancy losses [2]. Given that incomplete fetal maturation is associated with postpartum death, it is important to ensure that delivery is at full term, as fetal maturation is not reached until the final days of gestation [3].

Fetal lung immaturity is a cause of unfavorable early postpartum outcomes. Much of what is known in regard to its complications is derived from experience with respiratory distress syndrome (RDS), in human infants [4]. RDS is caused by underproduction of surfactant due to immaturity of type II pneumocytes, which results in poor function of immature alveoli that normally continue to develop throughout post-natal life [5]. Puppies born by C-section are reported to have a higher mortality rate than puppies born by vaginal delivery [6]. The incidence of RDS in puppies is unknown, but a recent study found a correlation between obstetrical conditions and respiratory performance [7].

In veterinary medical practice, ultrasonography (US) is central to antenatal assessment of viability, anatomy, and gestational age of the fetus [8]. The ultrasonographic appearance of foetal lungs and liver have already been described in puppies [3,8], with a first clear distinction between abdomen and thorax around day 34–36, and lungs being more hyperechoic than liver between days 38–42 [3].

At present, the utility of US to assess fetal pulmonary development is beginning to be understood in humans. For example, sonographic histogram analysis of fetal lung and liver tissues show promising results for determination of fetal lung maturity and prediction of RDS [9,10]. Additionally, lung-to-liver ratio of the grey-level-histogram-width (GLHW) show a moderate diagnostic accuracy in the prediction of RDS but the combination of several different parameters, including: lung-to-liver ratio of the GLHW,

* Corresponding author.
*E-mail address:* tommaso.banzato@unipd.it (T. Banzato).

http://dx.doi.org/10.1016/j.theriogenology.2017.03.011
0093-691X/

gestational age and estimated fetal weight increase diagnostic accuracy [9].

Quantitative analysis of ultrasonographic images has been used to establish normal canine hepatic echogenicity [11], and normal feline renal echogenicity [12]. Moreover, recently the *ex-vivo* correlation between renal cortical echogenicity and renal histopathology [13], and between hepatic echogenicity and hepatic histopathology in the canine and feline species [14], has been published.

The hystological pulmonary development in the dog has been described [5]and divided in four periods: 1) pseudoglandular phase, occurring between 35th and 48th days of gestation; 2) canalicular phase, from 49th and 56th days of gestation; 3) saccular phase, around day 57th and 60$^{th}$, and 4) alveolar phase, occurring in early post-natal life. It is during the canalicular phase that pneumocytes start their development and maturation leading to surfactant production.

The aim of this work was to explore the possibilities of quantitative analysis of ultrasonographic images in the assessment of canine fetal pulmonary maturity. Specifically, we investigated the relationship between fetal pulmonary maturity and echogenicity during three maturation phases – pseudoglandular, canalicular, and saccular – in normal canine pregnancies. Additionally, we assessed pulmonary maturity by quantitative sonographic analysis.

## 2. Materials and methods

### *2.1. Animals*

Twelve client-owned pregnant bitches (2 Jack Russell Terrier, 3 Boxer, 1 Australian Shepherd Dog, 1 Collie, 2 Flat coated Retriever, 1 Samoiedo, 1 Golden retriever, 1 Bouvier des Flandres) presented from October 2015 to August 2016 to the Reproduction Unit of the Veterinary Teaching Hospital of the University of Padua for the estimation of the optimal breeding time were included in the study. All the bitches belonged to professional or amateur breeders and written consent was always obtained from the owners. The bitches were either naturally mated or artificially inseminated after determination of the optimal breeding time. The day of ovulation (day 0) was estimated to occur when the serum progesterone concentrations were between 4 and 10 ng/ml using a chemiluminescence method (CLIA, Immulite® 1000, Medical System, Siemens, UK) validated for the dog [15]. A complete clinical examination was performed before every sonogram. If any abnormality was detected during examination, the bitch was removed from the study. All the bitches were followed to delivery and any puppy abnormality in early post-natal life was recorded. Gestational age was retroactively calculated from the day of delivery.

### *2.2. Ultrasonographic procedures*

All of the sonograms were performed with a 4–8,5 MHz microconvex probe connected to a commercially available ultrasound (Z-One, Zonare Medical Systems Inc). The following settings were maintained throughout the scans: depth 6 cm, frequency 8,5 MHz, and gain 90. Time-gain-compensation settings were maintained in a neutral position. Tissue harmonics and compound imaging were used to enhance image quality. Serial sonograms were performed during three different periods corresponding to the timeframes of the pseudoglandular, canalicular, and saccular phases of lung development: days 40–48 (period 1), days 49–56 (period 2) and days 57–63 (period 3) of gestation [5]. The two caudal foetuses (one from each uterine horn) were imaged. Longitudinal and transverse scans of the lungs and the liver (Fig. 1) were performed for each foetus. Several images were acquired during in each scan.

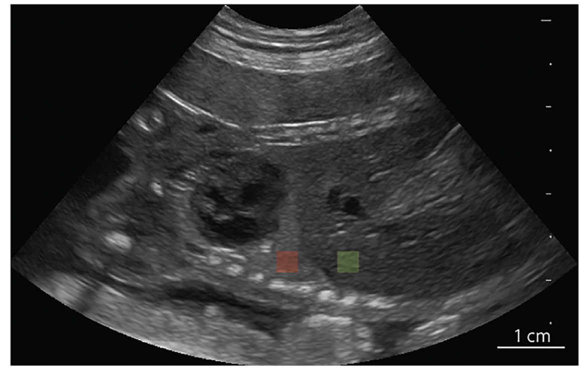


**Fig. 1.** Normal ultrasonographic image of foetal lung and liver in a longitudinal scanning plane. The red and green squares are the regions of interest (RoIs) used in Mazda to calculate the histogram parameters. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

### *2.3. Image analysis*

A single ultrasound image of each foetus was chosen for each time period based on image diagnostic quality. All the analysis were performed either on a longitudinal or on a transverse scan. The images were imported, in a bitmap format, into dedicated freeware software (MaZda v4.6). A 30-pixel region of interest (ROI) was placed in the lungs and in the liver of each image (Fig. 1). Shadowing artefacts caused by the fetal ribs were carefully avoided. Histogram based parameters mean grey level (MGL) and the standard deviation of the histogram (SDH) were calculated. The ROIs of lungs and liver were placed approximately at the same depth. To minimize the effects of both extreme brightness and contrast variation on the outcome of the analysis, grey level histogram normalization was calculated with MaZda by rescaling the histogram data to fit $\mu \pm 3\sigma$ ($\mu$: grey level mean; $\sigma$: grey level standard deviation).

### *2.4. Statistical analysis*

The average value of MGL and SDH calculated in the lungs and in the liver of the two caudal-most foetuses of each breeding bitch was considered in the analysis. All analyses were performed though commercially available software (IBM Corp. Released 2011. IBM

**Table 1**
Descriptive statistics of mean grey level (MGL), and standard deviation of the histogram (SDH) calculated in the foetal lungs for the three time periods considered, expressed as median with the limits of the overall range.

| Ultrasonographic parameter Foetal Lungs | Time periods | | |
|---|---|---|---|
| | 40-48 days | 49-56 days | 57-63 days |
| MGL | 84.58 (71.57–106.74) | 109.58 (76.94–120.70) | 108.75 (89.61–124.79) |
| SDH | 10.74 (9.14–11.7) | 10.25 (8.09–11.69) | 11.15 (9.33–12.1) |