RESEARCH ARTICLE
Open Access

Journal of Veterinary and Animal Sciences
ISSN (Print): 0971-0701, (Online): 2582-0605
https://doi.org/10.51966/jvas.2024.55.2.360-365

Relationship between the sonographic foetal lung-to-liver ratio of mean grey level and progesterone concentration in canine late gestation#

Roshni Ravindranath[1], K. Magnus Paul [2*], K. Promod[3], C.P. Abdul Azees[4] and N.S. Jinesh Kumar [5]
Department of Animal Reproduction, Gynaecology and Obstetrics
College of Veterinary and Animal Sciences, Pookode, Wayanad-673 576,
Kerala Veterinary and Animal Sciences University
Kerala, India

Citation: Ravindranath, R., Paul, M.K., Promod, K., Azees, C.P.A. and Jinesh Kumar, N.S. 2024. Relationship between the sonographic foetal lung-to-liver ratio of mean grey level and progesterone concentration in canine late gestation. *J. Vet. Anim. Sci.* **55**(2):360-365
DOI: https://doi.org/10.51966/jvas.2024.55.2.360-365

Received: 16.10.2023 *Accepted:* *Published: 30.06.2024*

Abstract

A study was conducted to perform the quantitative analysis of canine foetal lung and liver sonographic images in the late term of gestation (58-67 days) and to evaluate its association with serum progesterone concentration. Thirty-two pregnant bitches were selected for the study. Lung development of foetus occurs in three different stages viz the pseudo glandular (40-48), canalicular (49-56 d) and saccular phase (57-63 d) of pregnancy. Lungs of the foetus at the time of parturition are in saccular phase of development and the alveolar phase occurs in the neonatal period. In the present study, sonographic changes occurring in the lung during the saccular phase of foetal lung development was evaluated. Mean grey level of the foetal lung and liver sonographic images were estimated using dedicated image analysis software Image J. The lung-to-liver ratio (LLR) of mean grey level (MGL) was recorded. Corresponding progesterone concentrations were also estimated for each animal. Correlation statistical analysis revealed a significant correlation between LLR of MGL and progesterone concentration in the animals selected for the study. LLR of MGL is a prognostic indicator of foetal survival and since LLR of MGL and progesterone are positively correlated, it can be used as an indicator of foetal maturity in place of progesterone concentration.

#Part of MVSc thesis submitted to Kerala Veterinary and Animal Sciences University, Pookode, Wayanad, Kerala

1. *MVSc Scholar*
2. *Assistant Professor, Department of Animal Reproduction, Gynaecology and Obstetrics, CVAS, Mannuthy*
3. *Professor and Head*
4. *Associate Professor*
5. *Assistant Professor, Department of Veterinary Surgery and Radiology, CVAS, Pookode*

**Corresponding author: magnus@kvasu.ac.in, Ph. 9400322175*

Keywords: Lung-to-liver ratio of mean grey level, ultrasonography, progesterone

Gestational period in canines is highly variable depending upon factors such as variable duration oestrus behaviour, multiple breeding and sperm viability in the female tract (Lopate, 2008). Neonatal death due to foetal immaturity is an unfavourable outcome after the birth. Ultrasonography is central to neonatal assessment around the peripartum period in veterinary obstetrics. Doppler ultrasonographic examination and bi-parietal diameter measurement are routinely performed during ultrasound scanning. The anatomical development of foetal structures is closely related to the gestation age. The lung and liver are the largest organs in the foetus that are not usually given much importance during ultrasound scanning. In humans, lung to liver ratio of the grey level histogram width is studied and utilised for the prediction of respiratory distress syndrome combined with gestational age (Maeda *et al*., 1999).

Assessment of gestational age and foetal maturation is essential in several situations. This could be due to the reason that the majority of cases are presented when there is insufficient or unavailable ovulation timing to accurately determine the due date. The progesterone concentration in late gestation between days 50 to 60 can range from 3 to 15 ng/mL (Concannon *et al*., 2001). Bonte *et al.* (2017) observed that progesterone values less than 2 ng/ml suggest that foetal maturation and parturition will occur within 18-36 hours. The aim of the present study was to explore the possibilities of quantitative analysis of the foetal lung and liver echogenicity change and its relation to the late-stage canine serum progesterone concentration.

Materials and methods

Selection of animals

The study population contained healthy, medium to large-sized bitches of various mesencephalic breeds (Rottweiler, German Shepherd, Labrador Retriever, Siberian Husky, Indian Spitz, Beagle, Cross-bred, Belgian Malinois and Shiba Inu) weighing between 13 to 35kg. Animals in first gestation to third parity were selected for the study. Mesencephalic female dogs presented to Teaching Veterinary Clinical Complex, Mannuthy and Pookode at the late term of gestation between 58 to 67 days as estimated using ultrasonography based on bi-parietal diameter (BPD) were considered for the present study.

Upon presentation of the animals, age, breed, parity, breeding dates, previous history of dystocia, etc. were recorded. The age of bitches included in the study ranged between one to six years. Physiological parameters like rectal temperature, heart rate, respiratory rate, colour of visible mucous membrane and capillary refill time were recorded.

Ultrasonographic examination of pregnant animals and image analysis

B mode ultrasonographic examination was performed in the animals using MyLab X8 Esaote portable scanner using a linear multifrequency transducer probe ranging 4-15 MHz frequencies. The foetal heart rate was recorded using the pulsed doppler mode and the viability of the foetus was assessed using the large curvilinear probe (3-11MHz). Foetal head was focused and the biparietal diameter (BPD) was measured using the digital caliper available in the scanner. The measurement was standardised by recording the distance when the parietal bones were aligned parallel to each other. Gestational age was calculated using standard formulas, GA (days) = (15×HD) +20 where GA is gestational age and HD is the head diameter (cm) (Nyland and Mattoon, 2002) and GA= (13.28× BPD) + 27.58 (BPD in cm) (Vinaykumar, 2020). Foetal lung and liver images were captured together in a frame during the scan (Fig. 1). The following settings were maintained throughout the scans: depth 65 mm, frequency 7-15 MHz (linear transducer), neutral time gain compensation and gain 90. Later the images were imported in a bitmap format into the image analysis software Image J (Fig. 2). Histogram-based parameter mean grey level along with the minimum and maximum grey levels were estimated on the foetal lung and liver regions on multiple regions and averaged.

Shadowing artifacts caused by foetal ribs were carefully avoided. A region of interest of 800 mm^2 was selected on both the regions coming in the same depth. All the analyses were conducted on either a longitudinal or a transverse scan. The lung-to-liver ratio of mean grey level was calculated from the image analysed data.

Blood collection and serum progesterone estimation

Whole blood was collected from the animals on the day of ultrasonographic examination. Serum was separated for estimating serum progesterone concentration by Chemiluminescence Immuno Assay (CLIA) using commercial CLIA kits (PROG).

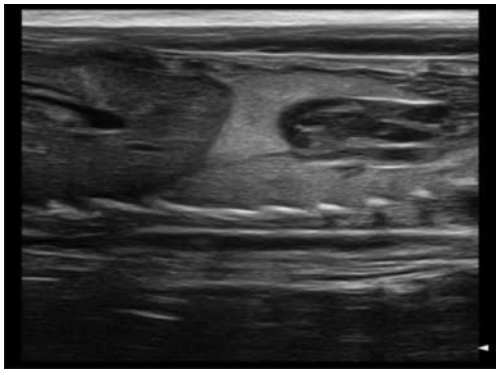

Fig. 1. Ultrasonographic image of pregnancy on day 60 of gestation, showing foetal lung and liver

Statistical analysis

The data obtained were tabulated and analysed statistically using the SPSS version 24.0 statistics software package. Normality of the collected data was statistically assessed by the Shapiro-Wilk test. The lung-to-liver ratio of mean grey level was found to be following normal distribution. Correlation between lung-to-liver ratio of mean grey level and serum progesterone levels was tested using Pearson correlation test.

Results and discussion

The sonographic foetal lung-to-liver ratio of mean grey levels and serum progesterone concentrations of a total of 32 pregnant bitches of nine different breeds presented for pregnancy ultrasound scanning were analysed and the following results were interpreted.

Ultrasonographic examination and image analysis

Ultrasonography enabled the estimation of gestational age by employing foetal biometric measurements and assessing the progression of foetal development. Additionally, it served to confirm viability and identify signs of foetal distress. Multiple measurements are collected when evaluating both foetal and extra-

Fig. 2. Analysis of sonographic image measuring the mean grey level, minimum and maximum grey values of foetus with gestational age around 60 days using Image J software.

foetal biometrics, and these measurements are then averaged throughout the study to reduce potential errors. The anatomical development of foetal structures is closely related to the gestation age.

Ultrasonographic examination of pregnant animals revealed a clearly visualisable foetus, with a clear distinction between the thoracic and abdominal organs. The foetal lung appeared brighter in the ultrasonogram than the liver during the period of examination ranging from 58 to 67 days of gestation. The echogenicity of the foetal lung, quantified in terms of the mean grey level was found to be higher than that of the liver during the period of examination. As the pregnancy progressed, the ultrasound appearance of the liver became brighter nearing to that of the lung but not exceeded and this change played a significant role in the observed variations in the LLR of MGL during the final stages of pregnancy under the study. A similar study was conducted by Thieme *et al*. (1983) to evaluate the sonographic characteristics of foetal lamb and they observed that the reflection of the lung is equivalent to the liver at term and greater than the liver reflection at mid-gestation. In the field of human medicine to achieve consistent and comparable results, the echogenicity of the foetal lungs is measured relative to that of the liver (Maeda *et al*., 1999). In this study, the measurement of the echogenicity in the liver and lung regions of the foetus as mean grey level was solely conducted for the purpose of comparison and was not intended to be utilised as an independent parameter.

Banzato *et al*. (2017) conducted a quantitative analysis of the sonographic foetal lung and liver images to assess the characteristics of the foetal lung and liver in a detailed manner. This analysis involved precise measurements and evaluations to gather quantitative data regarding the developmental progress of these organs through the advancement of gestational age. The measurement of mean grey level (MGL) in the lungs exhibited a noteworthy increase during the pseudoglandular phase, occurring between 40-48 days of pregnancy. This increase then stabilised and reached a plateau during both the canalicular phase (49-56 days of pregnancy) and the saccular phase (57-63 days of pregnancy). In the present study, the saccular phase (5-63 days of pregnancy) of foetal lung development was studied and the mean grey levels of lung and liver were obtained.

The liver is chosen as the appropriate reference organ for comparison because of its proximity to the lungs and the specific anatomical characteristics it possesses (Sohn *et al*., 1991). Since measurements of both the lungs and the liver were obtained at the same depth in the present study, it is important to note that the MGL of the regions of interest (ROIs) selected in both lung and liver is influenced by the same set of factors. This consistency in measurement depth helps ensure that any variations observed in MGL can be more reliably attributed to the specific characteristics of the organs themselves rather than differences in measurement location or depth.

Ultrasound examination is incapable to directly measure any of the biochemical aspects related to foetal maturity or provide direct histological details concerning the development of the foetal organs like lung. It is a valid assumption that both morphological and biochemical transformations can influence the way ultrasound waves scatter and propagate within the foetal organs. As a result, these changes may manifest as alterations in the textural appearance of sonograms (Prakash *et al.,* 2002). Likewise, in the present study, our aim was to investigate the correlation between late-term endocrinological shifts and the alterations in ultrasound echogenicity of foetal lung and liver.

Serum progesterone levels exhibit their highest concentrations between days 15 and 30, with the potential to reach peaks ranging from a maximum of 80 ng/ml to a minimum of 15 ng/ml. As pregnancy progresses to the later stages, around days 50 to 60, progesterone levels decreases, ranging from as high as 15 ng/ml to as low as 3 ng/ml. Notably, in the critical 24-hour period leading up to the onset of labour, progesterone concentration experiences a decline, decreasing from approximately 4 to 5 ng/ml to levels near or even below 2 ng/ml (Concannon *et al.,* 2001). The serum progesterone concentration declines during the

Table 1. Correlation between lung-to-liver ratio of mean grey level and progesterone concentration

Number	Variables		Correlation	P-value
n=32	LLR of MGL	Progesterone Concentration ng/ml	0.669	<0.001**

*** Significant at 0.01 level*

late term of gestation, and this fall in progesterone concentration was studied and correlated with the quantitative sonographic parameter LLR of MGL. The variables were positively correlated and the correlation coefficient was 0.669 (Table 1). This denotes that as the progesterone concentration decreases, the LLR of MGL also tends to decrease. A reduction in progesterone concentration is a crucial factor in the initiation of the process of parturition.

As the pregnancy progresses and the gestational age advances, foetal organs undergo maturation, accompanied by a series of biochemical and physiological transformations within these organs. These changes manifest quantitatively in the sonographic echogenic properties observed during ultrasound examinations. This transformation can be comprehended by examining the corresponding fluctuations in the progesterone levels of the pregnant dam. In this context, when we observe changes in the foetal organs, it can be correlated with alterations in the progesterone profile of the pregnant mother. These parallel changes in both foetal development and maternal progesterone levels are interconnected and can provide insights into the developmental milestones and hormonal dynamics that occur during pregnancy.

The mean ± SE of serum progesterone concentration (ng/ml) of the animals (n=32) selected for the study was 6.83 ± 0.59 ng/ml, with an overall range of 2.37 to 14.65 ng/ml. The gestational age was calculated and the mean ± SE gestational age was 61.06 ± 0.46 days. The mean ± SE of the lung-to-liver ratio of mean grey level of the animals (n=32) under study was 1.59 ± 0.02, with an overall range of 1.26 to 1.93.

Conclusion

In the present study, a clear positive correlation between these echogenicity variations and the concentration of serum progesterone could be established**.** The lung-to liver ratio of mean grey level and the serum progesterone level varied significantly between the animals as the gestational age advanced. In this context, it is possible to establish a connection of foetal organ characteristic alteration like the quantitative variations in the mean grey level with variations in the progesterone concentration of the pregnant dam. These parallel transformations in both foetal development and maternal progesterone concentrations are interrelated, offering valuable insights into the developmental milestones and hormonal dynamics that take place towards the end of gestation. The lung-to-liver ratio of mean grey level (LLR of MGL) is a prognostic indicator of foetal maturity and better survival. Since the progesterone concentration and the LLR of MGL are positively correlated, it can be used as an indicator of foetal maturity and the non-invasive method of ultrasonographic examination can be employed in place of progesterone concentration. Exploring the textural characteristics of ultrasound in conjunction with biochemical tests will contribute to confirming the validity of the method and reducing potential errors.

Conflict of interest

The authors declare that they have no conflict of interest.

References

Banzato, T., Zovi, G. and Milani, C. 2017. Estimation of foetal lung development using quantitative analysis of ultrasonographic images in normal canine pregnancy. *Theriogenology*. **99**: 158-163.

Bonte, T., Del Carro, A., Paquette, J., Charlot Valdieu, A., Buff, S. and Rosset, E. 2017. Foetal pulmonary maturity in dogs:

Estimated from bubble tests in amniotic fluid obtained via amniocentesis. Reprod. Domest. Anim. 52: 1025-1029.

Concannon, P., Tsutsui, T. and Shille, V. 2001. Embryo development, hormonal requirements

and maternal responses during canine pregnancy. *J. Reprod. Fertil.* **57**: 169-179.

Lopate, C. 2008. Estimation of gestational age and assessment of canine foetal maturation

using radiology and ultrasonography: a review. *Theriogenology*. **70**: 397-402.

Maeda, K., Utsu, M., Yamamoto, N., and Serizawa, M. 1999. Echogenicity of foetal lung and liver quantified by the grey-level histogram width. *Ultrasound Med. Biol*. **25:** 201-208.

Nyland, T.G. and Matoon, J.S. 2002. Small animal diagnostic ultrasound (2nd Ed.). W.B. Saunders Company, Philadelphia, pp. 231-249.

Prakash, K.B., Ramakrishnan, A.G., Suresh, S. and Chow, T.W. 2002. Foetal lung maturity analysis using ultrasound image features. *IEEE Trans. Inf. Technol. Biomed*. **6**: 38-45.

Sohn, C., Stolz, W. and Bastert, G. 1991. Diagnosis of foetal lung maturity by ultrasound: a new method and first results. *Ultrasound Obstet. Gynecol.* **1**: 345-348.

Thieme, G.A., Banjavic, R.A. and Johnson, M.L. 1983. Sonographic identification of lung maturation in the foetal lamb. *Invest. Radiol.* **18**: 18-26.

Vinaykumar, R.H. 2020. Development of a model for predicting whelping date in small breeds of dogs. *M.V.Sc. thesis*, Kerala Veterinary and Animal Sciences University, Pookode, 87p. ■