

Qualitative and quantitative ultrasound attributes of maternal-foetal structures in pregnant ewes

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Contents

The aim of this study was to examine foetal organs and placental tissue to establish a correlation between the changes in the composition of these structures associated with their maturation and the ultrasonographic characteristics of the images. Twenty-four pregnant ewes were included in the study. Ultrasonography assessments were performed in B-mode, from the ninth gestational week until parturition. The lungs, liver and kidneys of foetuses and placentomes were located in transverse and longitudinal sections to evaluate the echogenicity (hypoechoic, isoechoic, hyperechoic or mixed) and echotexture (homogeneous and heterogeneous) of the tissues of interest. For quantitative evaluation of the ultrasonographic characteristics, it was performed a computerized image analysis using a commercial software (Image ProPlus®). Mean numerical pixel values (NPVs), pixel heterogeneity (standard deviation of NPVs) and minimum and maximum pixel values were measured by selecting five circular regions of interest in each assessed tissue. All evaluated tissues presented significant variations in the NPVs, except for the liver. Pulmonary NPVmean, NPVmin and NPVmax decreased gradually through gestational weeks. The renal parameters gradually decreased with the advancement of the gestational weeks until the 17th week and later stabilized. The placentome NPVmean, NPVmin and NPVmax decreased gradually over the course of weeks. The hepatic tissue did not show echogenicity and echotexture variations and presented medium echogenicity and homogeneous echotexture throughout the experimental period. It was concluded that pixels numerical evaluation of maternal-foetal tissues was applicable and allowed the identification of quantitative ultrasonographic characteristics showing changes in echogenicity related to gestational age.

1 | INTRODUCTION

Intrauterine diagnosis of foetal maturation and development are essential to establish means to detect intercurrents during pregnancy in animals by establishing prenatal therapies, which increase the chances of foetal survival (Canello et al., 2015).

Given the importance of gestational evaluation, in medicine, foetal maturity is assessed by the study of tissues echotexture

using ultrasound examinations (Prakash, Ramakrishnan, Suresh, & Chow, 2002) and diagnosis of foetal diseases (Moeglin, Talmant, Duyme, & Lopez, 2005). Specifically in ovine, due to the impossibility of rectal palpation, the ultrasonography offers diagnostic information and important obstetric evaluation (Oliveira, Feliciano, & Vicente, 2014).

The gestational evaluation of sheep aims to ensure the proper evaluation of gestational development and birth of healthy animals,

with great conditions of development and weight (Fthenakis et al., 2012), estimate the foetal maturity on different stages of development, besides contributing to the increase in reproductive efficiency, in order to facilitate the early adaptation of the management to the new needs of the pregnant female (Aissi, Alloui, Slimani, & Touri, 2008; Davidson & Baker, 2009).

The use of ultrasound is already well established as a viable and non-invasive way to evaluate fetuses and its tissues, and a promisor alternative for the pulmonary maturity estimation. In humans, Podobnik, Brayer, Ciglar, Duić, and Podgajski (1996) analyzed echotextural attributes of the placenta, foetal liver and lung correlating the lung values with foetal maturity. Quantitative analysis of ultrasonography images has been used to establish normal canine hepatic echogenicity (Ivancic & Mai, 2008), and normal feline renal echogenicity (Drost, Henry, Meinkoth, Woods, & Lehenbauer, 2000).

We hypothesize that morphophysiological changes in maternal-foetal tissues during the gestational process in sheep can be verified by qualitative and quantitative ultrasound findings, assisting to monitor the development of these tissues. The aim of this study was to describe the sonographic characteristics of foetal pulmonary, renal and hepatic structures and placentomes during pregnancy in sheep, to establish qualitative and quantitative ultrasound patterns that can be correlated to physiological events of the gestational process of these females.

2 | MATERIAL AND METHODS

This study was conducted following the approval of the Animal Ethics and Welfare Committee of the School of Agricultural and Veterinary Sciences, Universidade Estadual Paulista, Jaboticabal, Brazil (protocol nº 10.387/16).

Twenty-four adults (2–5 years; 40–60 kg), healthy (normal physical, obstetrical and haematological examination and without history of reproductive diseases) Santa Inês ewes were selected for this study, from Animal Reproduction Department of the Universidade Estadual Paulista. These animals were kept in an experimental sheepfold, fed with corn silage, balanced commercial concentrate, mineral salt and water ad libitum.

The selected animals were submitted to an oestrous synchronization protocol: Day-0 intravaginal device containing progesterone 0.33 g (Eazi breed CIDR®, Pfizer, New Zealand) was applied; Day-5 cloprostenol 37.5 µg (Sincrocio®, Ourofino, Cravinhos, SP, Brazil) and equine chorionic gonadotrophin 300 IU (Novormon® Coopers MSD, Brazil) were IM injected; Day-6 intravaginal device was removed. After 24 hr, ewes were allocated for 72 hr with a breeder and the day of mating was considered as pregnancy day 0 and 21 days later, transrectal ultrasonography (Mylab ESAOTE, Italy—transducer of 5.0 a 7.5 MHz) evaluation was performed for gestational diagnosis.

Once confirmed pregnancy, the animals were brought to the ultrasound laboratory every day trying to adapt them to manage

and reduce stress. The abdominal area was clipped to facilitate ultrasound assessment. Weekly from the 9th (57–63 days) until parturition week, (145–152 days) the animals were brought to ultrasound laboratory and maintained in quadrupedal station for transabdominal ultrasonography scan, which was performed with the ACUSON S2000® (Siemens®, Munich, Germany) ultrasound system, equipped with convex multifrequential transducer (4C1®; 4–4.5 MHz; Siemens®, Munich, Germany).

Ultrasound exams revealed the pulmonary, renal and hepatic structures of the concepts and placentomes of females, in transverse and longitudinal sections, for echogenicity evaluation (hypoechoic, isoechoic, hyperechoic or mixed) and echotexture (homogeneous and heterogeneous) of these tissues. All images were obtained at constant settings of the ultrasound scanner for overall gain, near and far gains and focal points.

Posteriorly, B-mode sonograms containing the largest cross-sectional area of organs were saved as digital images (*) jpeg and subsequently computer-assisted analyzed by image analytical program (Image ProPlus, Media Cybernetics Inc., San Diego, CA, USA). The software assigns values from 0 (black colour) to 255 (white colour) and provides an indicator of tissue echogenicity. A primary interest area was defined by addressing the total parenchyma of evaluated organs and later, multiple regions of interest (ROI) spots were designed, measuring 20 mm in diameter each (Figure 1) to calculate mean, maximums, and minimums numerical pixel values (NPVs) and pixel heterogeneity (Heterog) as standard deviation NPVs values (SD–NPVs) from each interest area. For ROI selection special care was taken to exclude all reflection artifacts and non-parenchymal tissue.

Statistical analyzed was performed using software R® (R Foundation for Statistical Computing; Vienna, Austria). All data were analyzed to define normal residuals distribution (Shapiro–Wilk test) and homoscedastic variance (Barlett test). Actual or transformed measurements were compared between gestational weeks by analysis of variance (ANOVA) in a randomized block design (animals), when differences were significant, orthogonal contrast models (linear, quadratic and cubic) were tested trying to explain the sonographic parameters changes on time. The significance for all tests was set at 5% and results are expressed as mean ± standard deviation (SD).

3 | RESULTS

All animals had a normal gestational process, without obstetric complications or foetal abnormalities. The mean gestation time was 148 ± 1.8 days, with 16 singleton, seven twin and one triplet gestations, resulting in 33 healthy lambs.

3.1 | Lung

Pulmonary tissue presented homogeneous echotexture during all evaluated gestational weeks (GW) and, qualitatively, from 9th to

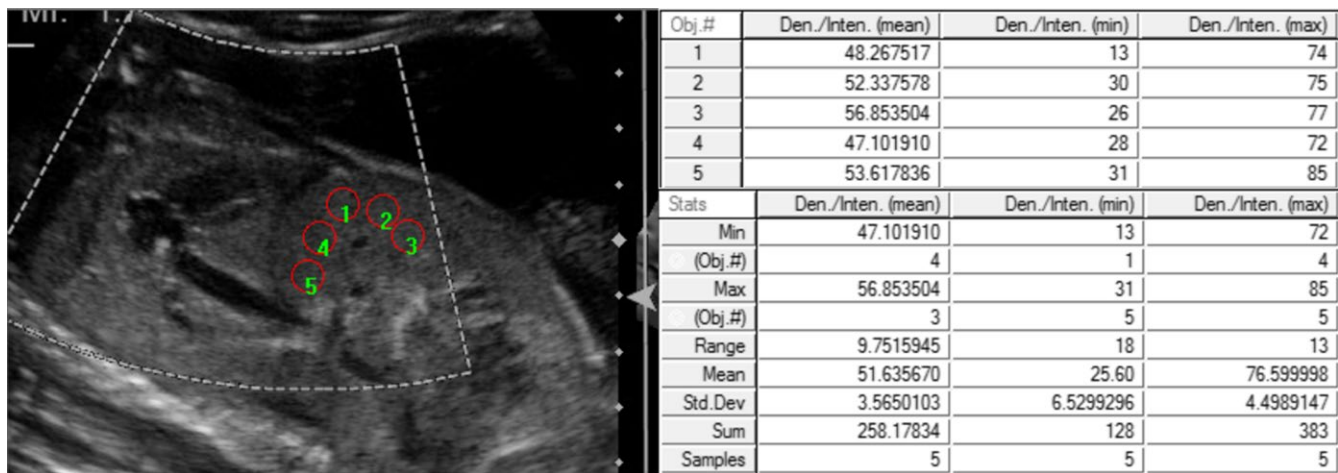


FIGURE 1 Longitudinal ultrasound image of ovine foetus with demarcation of regions of interest (ROIs—red) in hepatic tissue for the quantitative evaluation of tissue echogenicity using ImageProPlus software

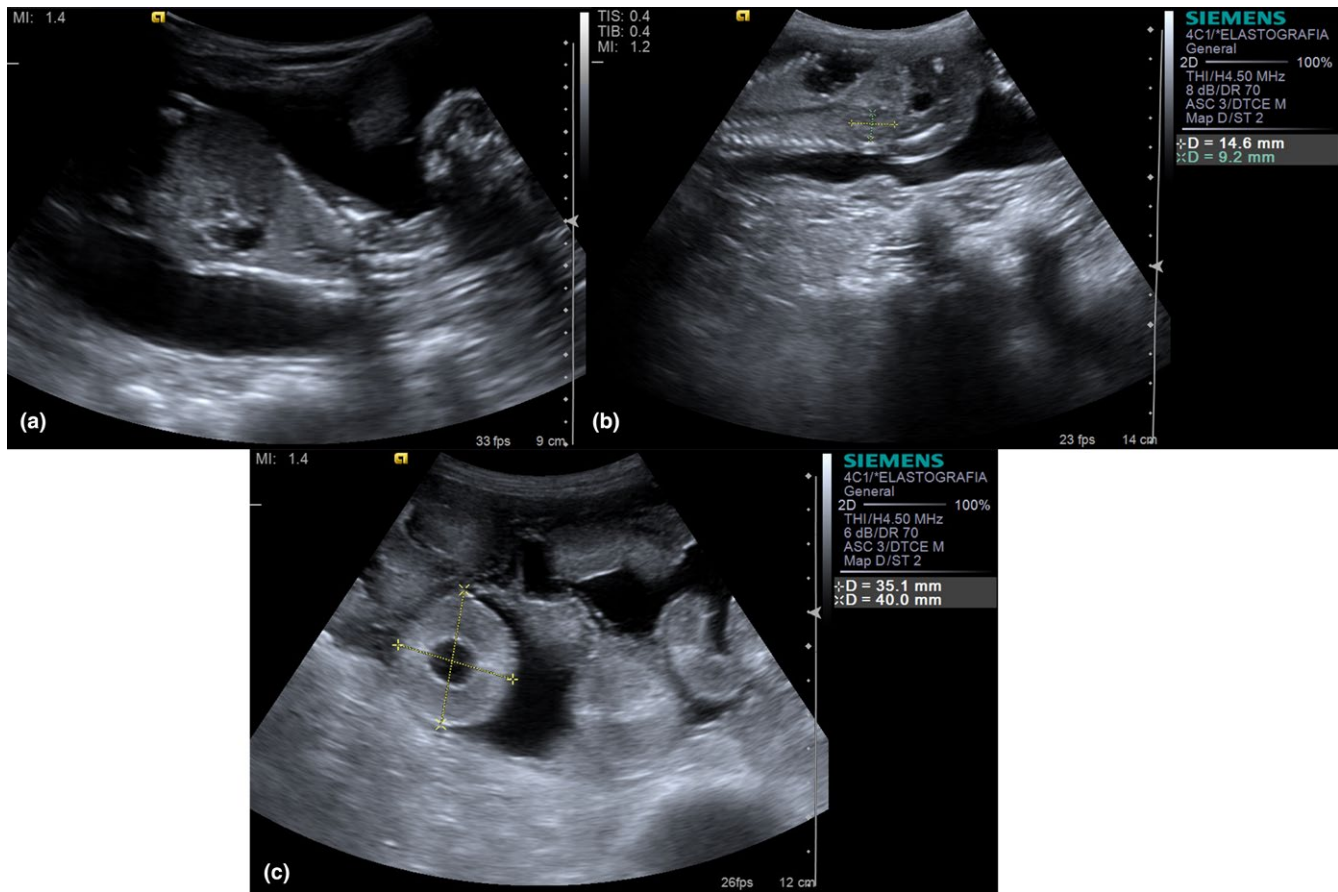


FIGURE 2 An ultrasonography image of ovine foetus (a) lung and liver on 10th gestational week (b) kidney on 11st GW (c) placentomes on 10thGW

11th GW it was hyperechoic in relation to the foetal liver (Figure 2a), with a gradual decrease in echogenicity throughout the gestational process, showing isoecogenicity with hepatic tissue (Figure 3a) from the 14th GW, a characteristic maintained until parturition.

The values for NPVmean, NPVmin and NPVmax decreased gradually with the advancement of gestational age (Figure 4a), and this

effect explained by a linear model ($p < .0001$; $R^2 = .8489$; 0.9181 ; 0.8502 , respectively). Mean NPV values ranged from 131.24 ± 22.44 to 64.98 ± 17.53 in the first and last evaluated weeks, respectively. NPVdesv and Heterog were constant during the evaluated period ($p = .2351$, $.4568$ respectively). There was no effect of foetal numbers on any of these parameters ($p > .05$) (Table 1).



FIGURE 3 An ultrasonography image of ovine foetus on 20th gestational week (a) lung and liver (b) kidney (c) placentomes

3.2 | Liver

The foetal hepatic tissue did not present variations in echogenicity and echotexture during the evaluated weeks, being homogeneous throughout the experimental period (Figures 2a and 3a).

NPVmean, NPVmin, NPVmax, NPVdesv and Heterog were found to be constant during the assessed period ($p = .29871$, $.692$, $.1187$, $.2667$ and $.1919$, respectively). In singleton pregnancies, NPVmean and NPVmin were higher ($p = .0298$ and $.0018$, respectively) than double or triple pregnancies; however, the values for NPVmax, NPVdesv and Heterog had no effect related to the number of concepts ($p = .1062$, $.5699$ and $.7927$, respectively) (Figure 4b).

3.3 | Kidneys

The foetal kidneys were detected from the 10th GW, as isoechoic to the hepatic tissue structures (Figure 2b), and is not possible to visualize the corticomedullary differentiation at this moment. Throughout the GWs, specifically from the 12th, corticomedullary differentiation became evident (ratio 1:1), with hyperechoic areas

(renal pelvis), hypoechoic (cortex) and anechoic (medullary) distinct areas (Figure 3b).

The values of NPVmean, NPVmin, NPVmax decreased gradually from 10th to the 17th GW and subsequently stabilized (Figure 4c), corresponding to a quadratic regression model ($p < .0001$, $R^2 = .9514$, $.9276$ and $.8213$, respectively). Mean NPV values ranged from 84.10 ± 44.90 in the first to 39.14 ± 09.19 at last gestational week. NPVdesv and heterogeneity were constant during the evaluated period ($p = .0585$ and $.4396$, respectively). There was no effect of foetal numbers on any of these parameters ($p > .05$).

3.4 | Placentomes

The placentomes were visualized in the first evaluated gestational week (9th), as hyperechoic structures in relation to the adjacent tissues (Figure 2c) and, during the gestational gestation process, their echogenicity changed, becoming hypoechoic structures (Figure 3c) in relation to the adjacent tissues from the 18th GW. The echotexture remained homogeneous throughout the evaluations.

The values of NPVmean, NPVmin and NPVmax decreased gradually over the gestational weeks, an effect explained by a linear model

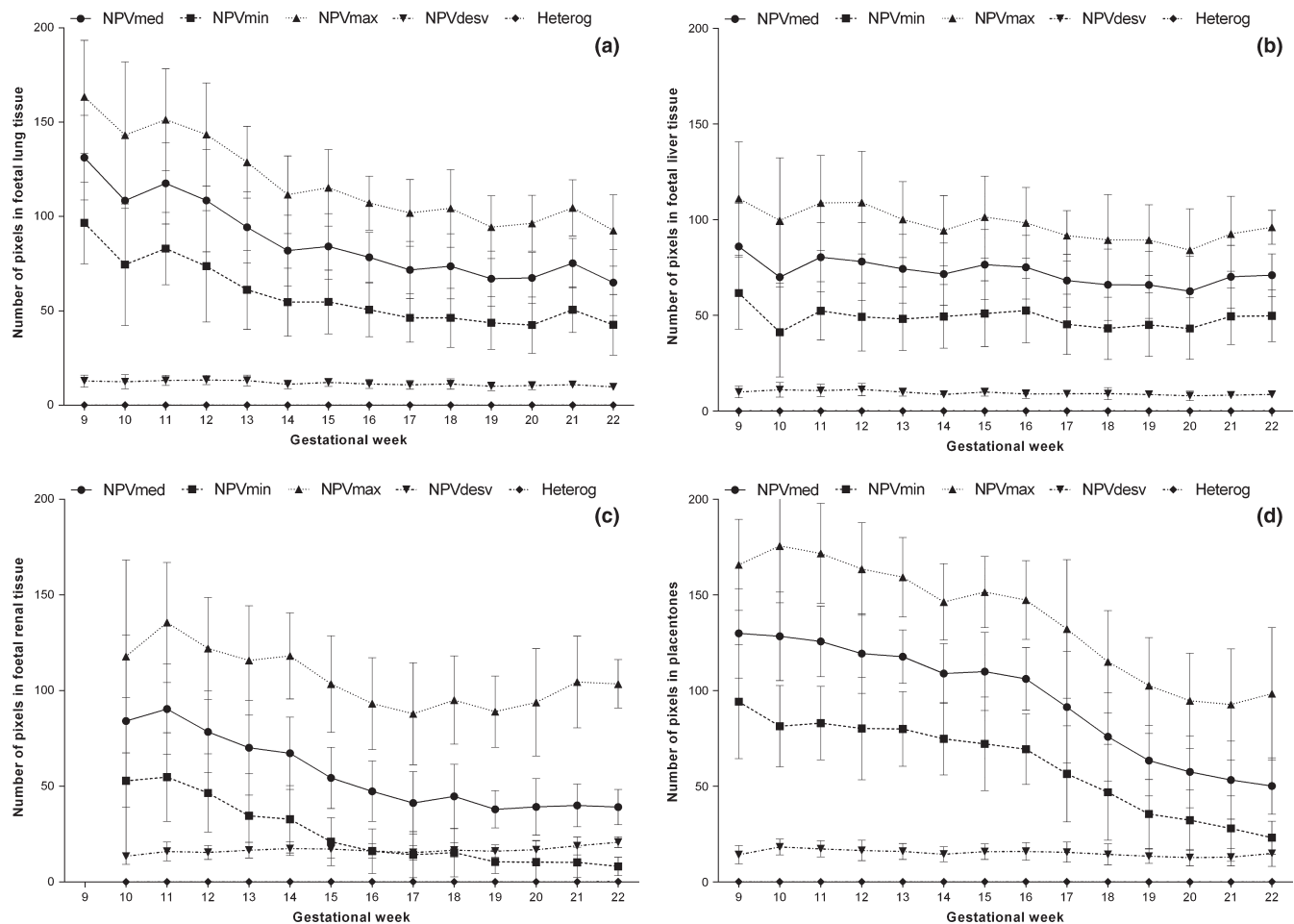


FIGURE 4 Graphic illustration of mean, maximums, minimum numerical pixel values (NPVs) and pixel heterogeneity as standard deviation NPVs values ($SD-NPVs$) evaluated from the 9th to the 22nd gestational week. (a) lung (b) liver (c) kidney (d) placentome

TABLE 1 Summary of mean quantitative echotexture numerical pixel values (NPVs); (mean $\pm$ SD. and ANOVA p -value); measured every week from 9th GW until parturition

Gestational weeks (GW)	Mean NPVs			
	Lung	Liver	Kidney	Placentome
9	131.24 $\pm$ 22.44	86.02 $\pm$ 22.49	N/D	129.94 $\pm$ 23.35
10	108.39 $\pm$ 34.54	69.95 $\pm$ 27.43	84.1 $\pm$ 44.9	128.43 $\pm$ 23.10
11	117.57 $\pm$ 21.54	80.42 $\pm$ 18.11	90.39 $\pm$ 23.69	125.69 $\pm$ 18.44
12	108.49 $\pm$ 27.14	78.14 $\pm$ 20.34	78.45 $\pm$ 21.39	119.37 $\pm$ 20.79
13	94.32 $\pm$ 18.94	74.34 $\pm$ 17.87	70.18 $\pm$ 24.76	117.77 $\pm$ 13.97
14	81.96 $\pm$ 18.78	71.59 $\pm$ 16.22	67.26 $\pm$ 18.84	108.94 $\pm$ 15.49
15	84.16 $\pm$ 17.40	76.50 $\pm$ 18.31	54.37 $\pm$ 15.99	109.97 $\pm$ 20.36
16	78.40 $\pm$ 13.15	75.19 $\pm$ 16.62	47.41 $\pm$ 15.79	106.18 $\pm$ 16.40
17	71.69 $\pm$ 15.13	68.19 $\pm$ 13.80	41.31 $\pm$ 16.36	91.39 $\pm$ 29.20
18	73.59 $\pm$ 17.22	66.02 $\pm$ 18.67	44.74 $\pm$ 16.77	75.88 $\pm$ 23.04
19	67.03 $\pm$ 14.52	65.89 $\pm$ 17.43	38.01 $\pm$ 9.81	63.44 $\pm$ 18.48
20	67.43 $\pm$ 13.52	62.58 $\pm$ 18.27	39.26 $\pm$ 14.73	57.57 $\pm$ 18.84
21	75.24 $\pm$ 13.23	70.13 $\pm$ 16.47	39.99 $\pm$ 11.16	53.26 $\pm$ 20.38
22	64.98 $\pm$ 17.53	71.00 $\pm$ 11.15	39.14 $\pm$ 9.19	50.16 $\pm$ 14.78
p -value	<.0001	.29871	<.0001	<.0001

GW, gestational weeks; N/D, no data available.

($p < .0001$, $R^2 = .9338$, $.9289$ and $.9216$, respectively). Mean NPV values ranged from 129.94 ± 23.35 to 50.16 ± 14.78 in the first and last evaluated weeks, respectively. The NPVdesv and the heterogeneity of placentomes were constant during the evaluated period ($p = .1877$ and $.7276$, respectively) and there was no effect of foetal numbers on any of these parameters ($p > .05$) (Figure 4d).

4 | DISCUSSION

The gestational ultrasound evaluation in sheep has shown to be applicable, practical and easy to perform, without any adverse effects observed for the maternal-foetal binomial. Both qualitative and quantitative maternal-foetal tissues analyzes were performed without difficulties, determining echogenic characteristics and numerical pixel values that correspond to the gestational phases development, which may help in the understanding of the maturity of ovine conceptus.

As well as in humans, pulmonary development in sheep can be divided into phases (embryonic, 0–40 days' gestation; pseudoglandular, 40–80 days; canalicular, 80–120 days; alveolar, 120 days to term (148 days) (Alcorn, Adamson, Maloney, & Robinson, 1981; Gnanaalingham et al., 2005). Considering the obtained results in the present study, it is possible to affirm that the observed modifications in the pulmonary echogenicity of the conceptus are directly related to the phases mentioned above, to the development of this tissue during the organogenesis.

Initially, the differentiation of lung tissue compared to other foetal tissues (ex. Liver) occurs from the 9th to the 11th GW, corresponding to the pseudoglandular phase. During the canicular phase of pulmonary development, a period that involves the formation of bronchioles, alveolar ducts, primitive alveoli, vascular tissue, type I and type II pulmonary cell differentiation, pulmonary surfactant proteins and alveolar-capillary barrier (Joshi & Kotecha, 2007), it was found a decrease in echogenicity and mean NVPs of foetal lung tissue from ovine foetuses. Probably, this change in echogenicity is related to the formation of the mentioned tissues, the beginning of the alveolar structures filling by the foetal lung fluid, responsible for keeping the spaces distended, limiting the entry of amniotic fluid and allowing the growth and structural maturation of this tissue (Drozdowska, Cousens, Finlayson, Collie, & Dagleish, 2016; Harding & Hooper, 1996; Hooper & Harding, 1995).

According to Prakash et al. (2002), changes in pulmonary echogenicity are characteristics that may be associated with foetal maturity in humans, a finding that corroborates the correlation observed between ultrasonographic findings and lung development phases in ovine foetuses. Additionally, our results demonstrate that the parameters evaluated can be used to determine the gestational age, adapting to a linear mathematical model and demonstrating that the foetal lung from the 14th gestational week becomes less and less echogenic until the date of birth.

In general, the hepatic tissue exhibits early embryogenic formation, which develops from endodermal shoots that arise in

the most caudal region of the embryonic gut, being regulated by a complex series of developmental events, including the specification of progenitor cells (Grundy, 2006; Nissim et al., 2016). The fact that the hepatic development occurs in the embryonic stage, from the 12th to the 34th day approximately (Jainudeen & Hafez, 2000), may explain the constant values and characteristics of the foetal liver's qualitative and quantitative findings during the gestational period. Then, ultrasonographic evaluation of this organ has a limited applicability in the study of gestational development as described in dogs (Banzato, Zovi, & Milani, 2017), however, the results of quantitative echogenicity evaluation may serve as a basis for studies of foetal developmental alterations, as these characteristics have been correlated with poor development in human neonates (Murao, 1991).

The renal structure and echogenicity changed markedly during 10th to 17th GW, mainly due to the corticomedullary differentiation. The development of the kidney occurs from the nephrogenic cord and the mesonephric channel, being divided into three phases originating successively, pronephros, mesonephros and metanephros (Serrão, Abranches, & Ferra de Sousa, 2003). It is suggested that this last phase comprises the period of differentiation of the renal regions of ovine concepts observed from the 12th week. Besides, the decrease in the echogenic attributes up to the 17th gestational week may be related to the onset and intensification of the foetal urine production and maturation of renal tissue. These findings may be clinically relevant for the study of gestational development, as urine is the main source of amniotic fluid during the second half of gestation and may indicate renal maturation and function (Gulbis, Gervy, & Jauniaux, 1998; Canelo et al., 2015). Decrease in renal echogenicity throughout gestation may be a feasible indicator of renal maturity, considering that the increase in foetal kidney echogenicity has been correlated with severe post-natal renal impairments in humans (Estroff, Mandell, & Benacerraf, 1991; Surányi, Retz, Rigo, Schaaps, & Foidart, 2001).

The placentomes are specialized non-glandular units composed of dense connective tissue that exhibit similar conformation and increase in size throughout the gestational process (Björkman, 1954; Hafez & Hafez, 2004; Aisen, 2008). The vascularization of this tissue is a significant and important feature for the gestational process, and the increase in blood flow in these placental structures throughout pregnancy is related to the nutritional requirements of the foetus and the proximity of parturition (Cunningham, 1999). In the present study, it was verified the reduction of placental echogenicity, especially from the 18th week of gestation, which may be correlated with the increase in the blood volume in these structures.

Finally, gestational ultrasonography in sheep is notably relevant to predict gestational age by means of foetal biometry (Doizé, Vaillancourt, Carabin, & Belanger, 1997; Bulnes, Moreno, & Sebastian, 1998), with highly correlated parameters such as crown-rump length and diameters of thoracic, biparietal and ocular orbits. These correlations are summarized in formulas that help this estimation of gestational age (Oliveira et al., 2014). Besides,

due to the observed correlation, it can corroborate with the pre-existing formulas to achieve a more accurate identification of the gestational age of ovine females, contributing to a more reliable ultrasound examination in the evaluation of gestation.

5 | CONCLUSION

The ultrasonographic qualitative characteristics of ovine foetal tissues (lung, liver and renal structures) and placentomes assist in the evaluation of gestational development in sheep and correlate with the gestational age of pregnant females.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

AUTHOR CONTRIBUTIONS

The designed study was performed by Priscila Del Aguila da Silva, Ricardo Andrés Ramirez Uscategui and Marcus Antonio Rossi Feliciano. In the experimental period, the samples were collected by Priscila Del Aguila da Silva, Ricardo Andrés Ramirez Uscategui, Victor José Correia Santos, Augusto Ryonosuke Taira, Renata Sitta Gomes Mariano, Mariana Garcia Kako Rodrigues, Ana Paula Rodrigues Simões, Marjory Cristina Maronezi, Michelle Lopes Avante and Marcus Antonio Rossi Feliciano. The results obtained were analyzed by Priscila Del Aguila da Silva, Ricardo Andrés Ramirez Uscategui and Marcus Antonio Rossi Feliciano. The preparation of the manuscript and its correction was carried out by Priscila Del Aguila da Silva, Ricardo Andrés Ramirez Uscategui, Wilter Ricardo Russo Vicente and Marcus Antonio Rossi Feliciano.

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