Journal of Equine Veterinary Science 141 (2024) 105162

Contents lists available at ScienceDirect

Journal of Equine Veterinary Science

journal homepage: www.elsevier.com/locate/jevs

Assessment of fetal development during mid and late term pregnancies by standard B-Mode ultrasonography in Shetland ponies

Lisa-Hélène Wagner, Jörg Aurich, Svenja Claaßen, Maria Melchert, Martim Kaps, Christine Aurich *

Clinical Center for Reproduction, Department for Small Animals and Horses, University for Veterinary Medicine (Vetmeduni) Vienna, Veterinärplatz 1 1210, Vienna, Austria

ARTICLE INFO

Keywords:
Horse
Fetus
Pregnancy
Fetal biometry
Transabdominal ultrasonography
Age prediction

ABSTRACT

Transrectal and transabdominal ultrasonography is an established method to monitor pregnancy, fetal growth and wellbeing in different species. Growth charts with multiple bio-morphometric parameters to estimate days of gestation and days before parturition exist in small companion animals, sheep and goats, riding type horses and large ponies but not in small horse breeds like Shetland ponies. The aim of this study was to apply fetal biometric assessment and detailed description of physiologic fetal development to mid and late term pregnancies in Shetland mares and to generate reference data for clinical practice and for future research. Fetal parameters were collected starting on day 101 of pregnancy in five Shetland mares. The fetal biometric parameters determined consisted of aortic diameter, eye diameter, combined rib and intercostal distance (CRID), stomach length and width and different heart morphology parameters in sagittal and frontal plane. Additionally, fetal activity and organ development in terms of differentiation and changes in echogenicity were recorded. Considering reliably assessable parameters, fetal CRID was the best predictor for gestational age with ± 13.6 days and fetal aortic diameter the most accurate for prediction of days until parturition with ± 16.2 days.

1. Introduction

In several domestic animal species, B-mode ultrasonography is routinely applied for monitoring of fetal growth and development and for assessment of fetal well-being [1–4]. Whereas in small companion animals, such as dogs and cats, transabdominal ultrasonography of pregnancy is easily applicable, this approach is more challenging in larger species. The major limitation in large animal species, including the horse, is the distance between the structure of interest and the ultrasound probe [5]. Especially during early gestation, when the pregnant uterus is not yet deviated towards the ventral abdomen, an increased penetration depth is required and ultimately results in a loss of detail resolution. In large domestic animals, a transrectal approach has thus been more widely applied, especially during mid-term pregnancy, but this approach allows only limited visualization of fetal structures when movements of the examiners arm are restricted by increasing fetal size in advanced pregnancy and accessibility of the cranial aspect of the uterus is also limited due to its enlargement [6]. Examination is therefore usually limited to the fetal skull including the eye when the fetus is in anterior position. A transrectal approach has been described in both riding-size type horses and smaller ponies [6–10]. Size of the fetal eye and diameter of the fetal aorta were suggested as good predictors for estimating fetal size and gestational age.

In smaller breeds, such as the Shetland pony, transrectal examination is associated with an increased risk for rectal lacerations caused by the decreased ratio of the examinators arm to rectum size of the mare and more frequent rectal straining [11–13]. The examination should thus be performed with caution [11]. For safety and animal welfare reasons, transabdominal ultrasonography might be preferred in very small horses. With advances in ultrasound technology, transabdominal imaging of pregnancy was improved also in large animals, allowing for visualization of fetal structures with satisfactory resolution. Transabdominal ultrasonography has therefore recently gained great interest for pregnancy monitoring in horses [2,8,9,14–18]. Estimation of gestational age with the aim to predict the onset of parturition is of major interest especially when the conception date is unknown.

For Quarter horses, Thoroughbreds, Warmbloods and large ponies, transabdominal sonography for pregnancy monitoring has been

* Corresponding author.
E-mail address: christine.aurich@vetmeduni.ac.at (C. Aurich).

https://doi.org/10.1016/j.jevs.2024.105162
Received 5 June 2024; Received in revised form 10 August 2024; Accepted 10 August 2024
Available online 12 August 2024

described in detail and growth charts for fetal development are available [8,9,18,19]. They include information on changes of the fetal biparietal diameter, fetal eye diameter, fetal aorta diameter and fetal femur length during gestation. Among those, measurements associated to the fetal eye and aorta diameter were most accurate for determination of gestational age and prediction of parturition. To the best of our knowledge, developmental data other than fetal eye measurements are not available for very small horse breeds like Shetland ponies. Prediction of the day of parturition is especially of benefit in ponies because they are commonly pasture-bred and the day of conception is therefore often unknown. Determination of gestational age could thus be helpful to meet the needs of the mare before foaling.

To the best of our knowledge, no information on either fetal biometry or fetal development assessed by transabdominal approach is available for Shetland ponies at present. The aim of this study was therefore to apply biometric assessment of fetal development to mid- and late term pregnancies in Shetland ponies and compile detailed data on physiologic organ development. We further investigated if biometric assessment can be used to estimate gestational age and predict the onset of parturition in this breed.

2. Materials and methods

2.1. Animals

The experiment was approved by the competent authority for animal experimentation in Austria (Federal Ministry for Science and Research, license number 2020–0.547.889). Five Shetland mares with a mean age of 12.0 ± 1.1 years (± SD) and a mean body weight of 216 ± 14 kg were available. Mares were kept in groups in outdoor paddocks with permanent access to a stable. They were fed hay twice daily and had permanent access to water and mineral supplements. After an initial breeding soundness examination to ensure reproductive health, mares were examined at five-day intervals. When a dominant follicle of >30 mm in diameter together with an endometrial edema was detected, mares were examined daily. When the dominant follicle reached a diameter of 35 mm, mares were inseminated with raw semen (500×10^6 progressively motile spermatozoa) from a fertile Shetland stallion at 48 h intervals until detection of ovulation (Day 0; i.e., disappearance of the preovulatory follicle). Pregnancy status was first determined via transrectal ultrasonography on day 10 (DP-6600 Vet; 6LE5Vs rectal transducer, 5–8 MHz, Mindray, Shenzhen, China) and further evaluated at regular intervals to ensure physiologic progression of pregnancy. From day 101 onwards, mares underwent repeated transabdominal ultrasonography at two-week intervals from day 101 to 280 and at 10-day intervals for the remaining pregnancy.

Mares were transferred to straw-bedded individual foaling stables (3.5 × 3.5 m) between days 305 and 310 of pregnancy. They were continuously monitored by video camera surveillance. All foalings were uneventful and no assistance was required. Foals underwent a routine examination protocol to ensure maturity and health. For assessment of adequate transfer of passive immunity, IgG concentrations were evaluated between 18 and 22 h after birth. The IgG concentration was above 800 mg/dL in all foals.

2.2. Transabdominal Ultrasonography

Transabdominal ultrasonography was performed using a C5–1s convex transducer with variable frequency (1.4-5.1 MHz; Mindray, Shenzhen, China). The initial settings were 5.0 MHz and a penetration depth of 10 to 15 cm. With increasing body size of the fetus, penetration depth and frequency were individually adapted to 1.3 to 3.0 MHz and 20 to 30 cm. To optimize image quality, the ventral abdomen was clipped in the region between the xyphoid and the mammary gland. Ethanol and ultrasonography coupling gel were applied liberally to the mares′ skin. For examination, the ultrasound probe was initially placed close to the mammary gland of the mare in dorso-median orientation and moved cranially in a serpentine pattern along the midline of the abdomen towards the xiphoid. Fetal ribs were used as landmark of the fetal thorax and the hypoechoic outline of the fetal stomach was used as a landmark of the abdominal cavity as previously described [2]. After fetal localization and orientation within the uterus were assessed, the whole fetus was visualized in a transversal plane (from head to tail or vice versa), followed by coronal (dorsal to ventral or vice versa) and sagittal plane (left to right or vice versa) imaging. No sedation or physical restraint of the mare was required at any time.

All examinations were videorecorded and stored for further evaluation. Video-recordings were transferred to a computer equipped with the MicroDicom DICOM Viewer (MicroDicom, Sofia, Bulgaria) for retrospective assessment of fetal biometric parameters.

2.3. Fetal biometric parameters

Diameter of the eye: A cross-section of the fetal ocular orbit was visualized. The anterior and posterior portions of the capsule of the lens and the osseous fundament of the eye served as anatomical landmarks. The video was frozen when the maximal cross-sectional diameter of the fetal eye was obtained. For standardization of the plane the lens was included in the frozen image. The length (diameter x) of the vitreous body was measured, by placing the curser line in a way that they divide the lens at a 90° angle, as previously described [2,8–10,20]. The width (diameter y) was measured from the cornea to retina, as described [10]. The mean diameter was calculated (Fig. 1).

Aorta diameter: The thoracic part of the aorta was visualized using a sagittal plane to produce an image with a longitudinal section. The video was frozen when the most anterior portion of the aorta (as it emerges from the heart) was visualized in systole (maximum dilation of the diameter of the aorta). The diameter of the aorta was measured by placing the two caliper cursors on the vessel margin (Fig. 2A) as described [9].

Combined rib and intercostal distance (CRID): The fetus was scanned in a frontal plane to visualize a cross-sectional image of the ribs in the caudal thoracic region during absence of fetal breathing movements (exertion of chest, movement of diaphragm). The distance between the cranial aspect of the third rib and the cranial aspect of the seventh rib was measured (Fig. 2B). To determine the combined distance of one rib and the following intercostal space (CRID), the measured distance was divided by 4, i.e. the number of ribs included [9].

Fetal heart parameters: For fetal heart biometry, the video was frozen at a longitudinal image (sagittal plane; four-chamber-view) of the heart. The diameter orthogonal to the septum (Fig. 3A) was defined as heart long axis width and the diameter parallel to the septum as heart long axis height (Fig. 3B [21]). Further, a section at the valve level of the heart (frontal plane) where the aortic valve, pulmonary valve, tricuspidal valve and bicuspidal valve could be visualized (Fig. 4) was used to measure the heart short axis 1 (craniocaudal) and 2 (laterolateral) in two perpendicular planes. The mean of the two measurements was calculated.

Fetal Stomach: The bean-shaped anechoic stomach is located in the cranial abdominal cavity caudally to the fetal liver and was visualized in a transversal plane of the fetus. Size of the stomach was measured in several axes. First the long axis was measured as the distance from the left to right aspect of the stomach (Fig. 5A). The two short axes were measured as the maximum dorso-ventral distance of the left and right part of the stomach, respectively (Fig. 5B, C).

2.4. Monitoring of fetal organ development

In addition to morphometric measurements, the development of fetal organs was monitored. The scoring system for intestinal development based on the criteria described by Gil et al. [22] and Agnew et al. [23] was applied as follows: Score 1: one uniform echogenic area caudal to

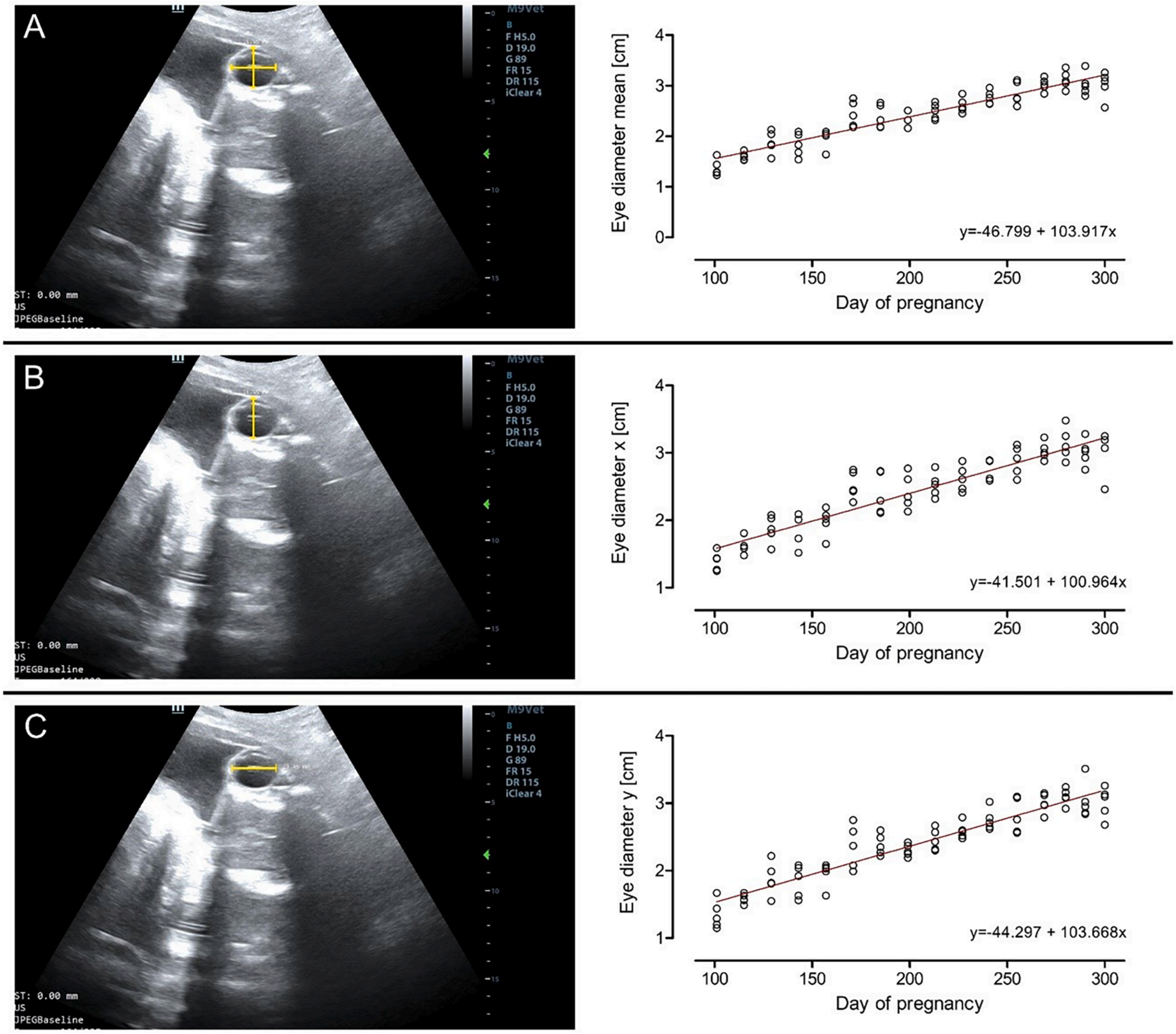

Fig. 1. Representative images shown in sagittal plane of the measurement of the (A) mean diameter of the fetal eye (yellow lines), (B) the diameter x of the fetal eye (yellow line) and (C) of the diameter y of the fetal eye (yellow line) together with the respective dot-plot of the individual fetal eye diameter mean, x, and y measured at the specific examination timepoints during pregnancy in Shetland pony mares (n=5). Linear regression line (red) and the concordant equation are given in the figure.

the fetal liver, no bowel segment and no intestinal contents visible, score 2: portions of bowel segments were better defined allowing visualization of the intestinal wall i.e., anechoic areas intermixed with multifocal normal intestinal wall (hypoechoic intestinal contents; Fig. 6A), peristalsis was absent, score 3: Bowel segments had clearly defined intestinal walls, anechoic areas were fewer when compared to phase 2; defined segments had mucosal intraluminal content, with better definition of the mucosal surface versus the intestinal wall (mixed echogenicity in all areas of intestinal contents visible; Fig. 6B). Peristalsis present in some intestinal portions, score 4: Identification of complete intestinal wall, visual distinction between the mucosal surface and the intestinal wall, ultrasonographic determination of wall layers (combination of mixed and hyperechoic intestinal content). Segmental dilatation of the bowel by intraluminal mucous and fluid content (Fig. 6C). Peristalsis in all segments of the bowel. The presence of the rugae of the fetal stomach (Fig. 6D) was documented.

The fetal gonads and kidneys were identified using the sagittal plane in the caudo-dorsal aspect in the abdominal cavity of the fetus. Echogenicity compared to the surrounding tissue was documented. Development of the renal pelvis was monitored. Measurement of the maximum diameter of the gonads and kidneys from cranial to caudal pole was performed if a clear differentiation from the adjacent surrounding tissues was possible. The day on which a clear differentiation of pulmonary and hepatic tissue as possible was documented for each pregnancy.

2.5. Fetal activity

Fetal actvitiy: Activity of the fetus was subjectively graded on a scale of 0 to 3 as described previously [16]. Briefly, score 0 was given if no movements were detected during the total examination time. Score 1 was given if slight movements were visible (<33 % of examination time), score 2 if the fetus was fairly active (>33-<66 % of the examination time) and score 3 if the fetus was very active with few or no quiet periods detectable (>66 % of the examination time).

2.6. Statistical Analysis

Statistical analysis was performed with the SPSS statistics package (version 29.0.1.0, IBM-SPSS, Armonk, NY, USA). Curve fit estimation was applied to test for linear, cubic, quadratic relation. Linear relation was determined to be the best fit for the biometric variables. A linear

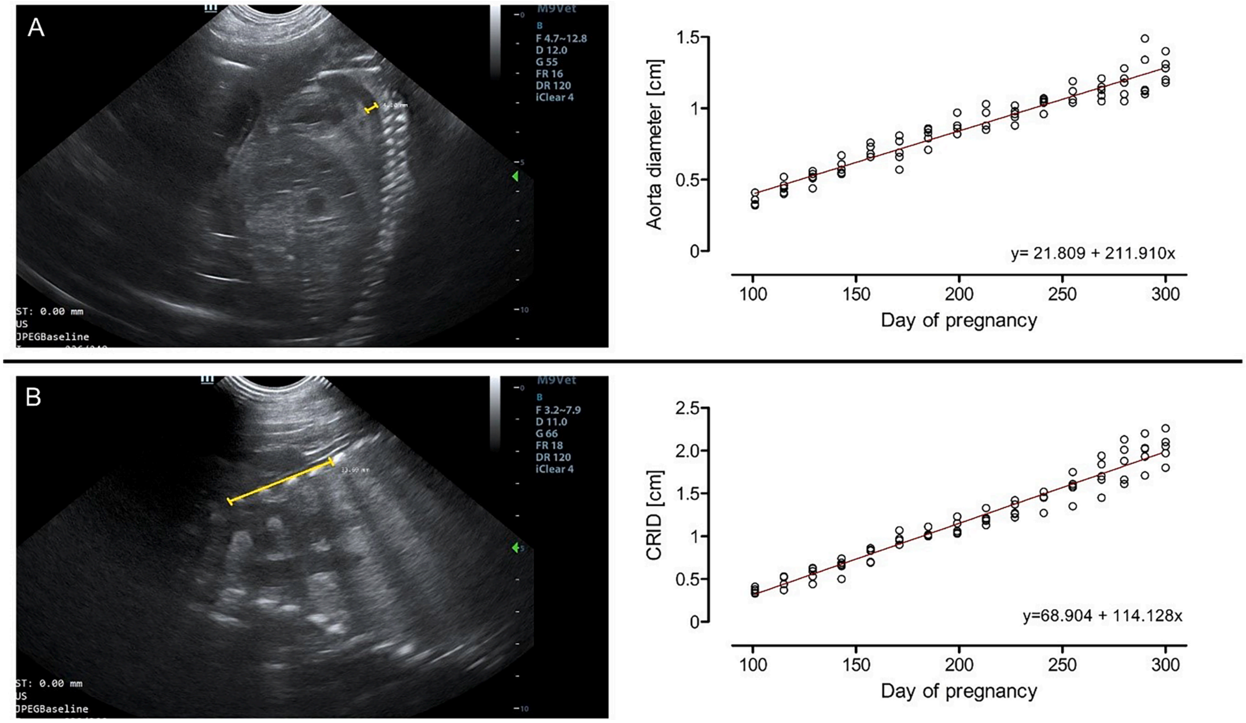

Fig. 2. Representative image of (A) the measurement of the diameter of the fetal aortic diameter (yellow line, sagittal plane) and (B) the measurement of the fetal combined rib and intercostal distance (CRID, yellow line, frontal plane). Dot-plot of the individual aorta diameter and CRID measured at the specific examination timepoints during pregnancy. Linear regression line (red) and the concordant equation are given in the figure.

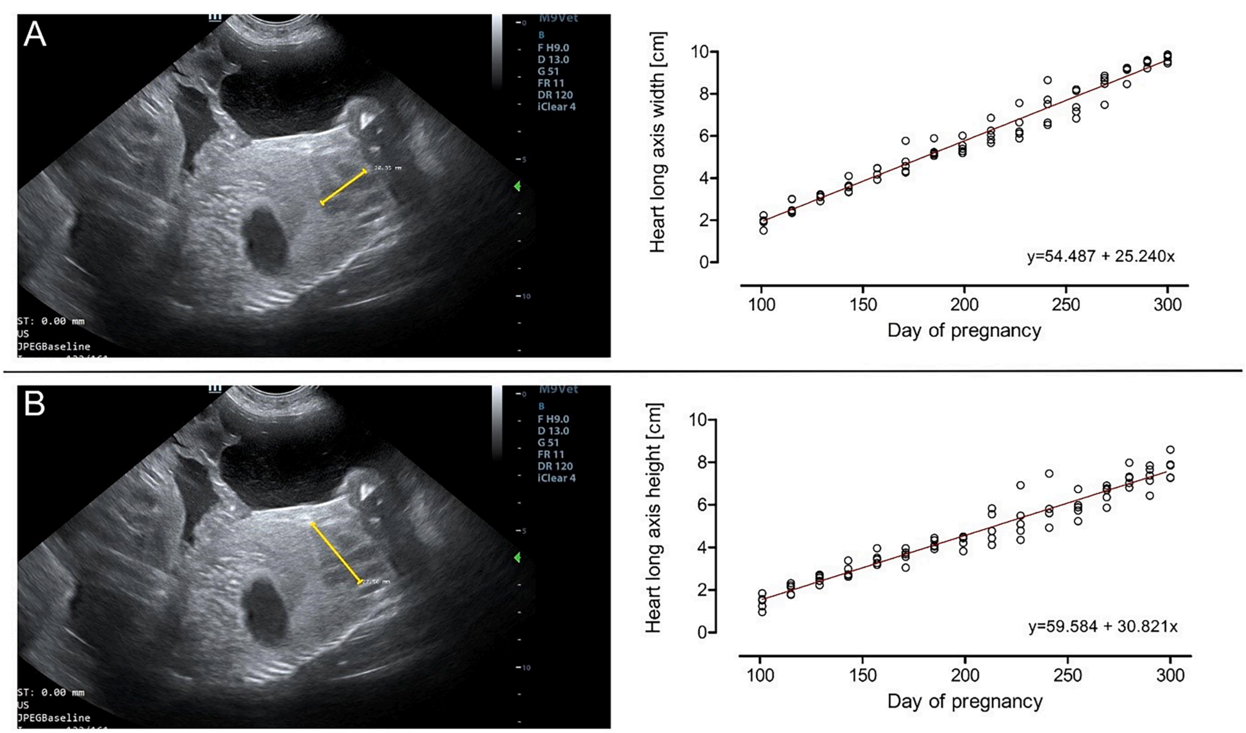

Fig. 3. Representative images for (A) the measurement of the fetal heart long axis width (sagittal plane) and (B) fetal heart long axis height (yellow line, sagittal plance) together with the respective dot-plots of the individual heart diameter short and heart diameter long measured at the specific examination timepoints during pregnancy in Shetland pony mares (n=5). Linear regression line (red) and the concordant equation are given in the figure.

regression model for each biometric variable was applied, with gestational age or days before parturition as outcome variables. Multiple regression analysis was not applied due to collinearity of the variables. Pearson's correlation was used to further characterize the relation of biometric variables with gestational age and days before parturition. Days before parturition was calculated by subtracting the day of examination from the total gestation length for each mare. For the parameters heart long axis height and width, 10 out of 80 values (12.5 %)

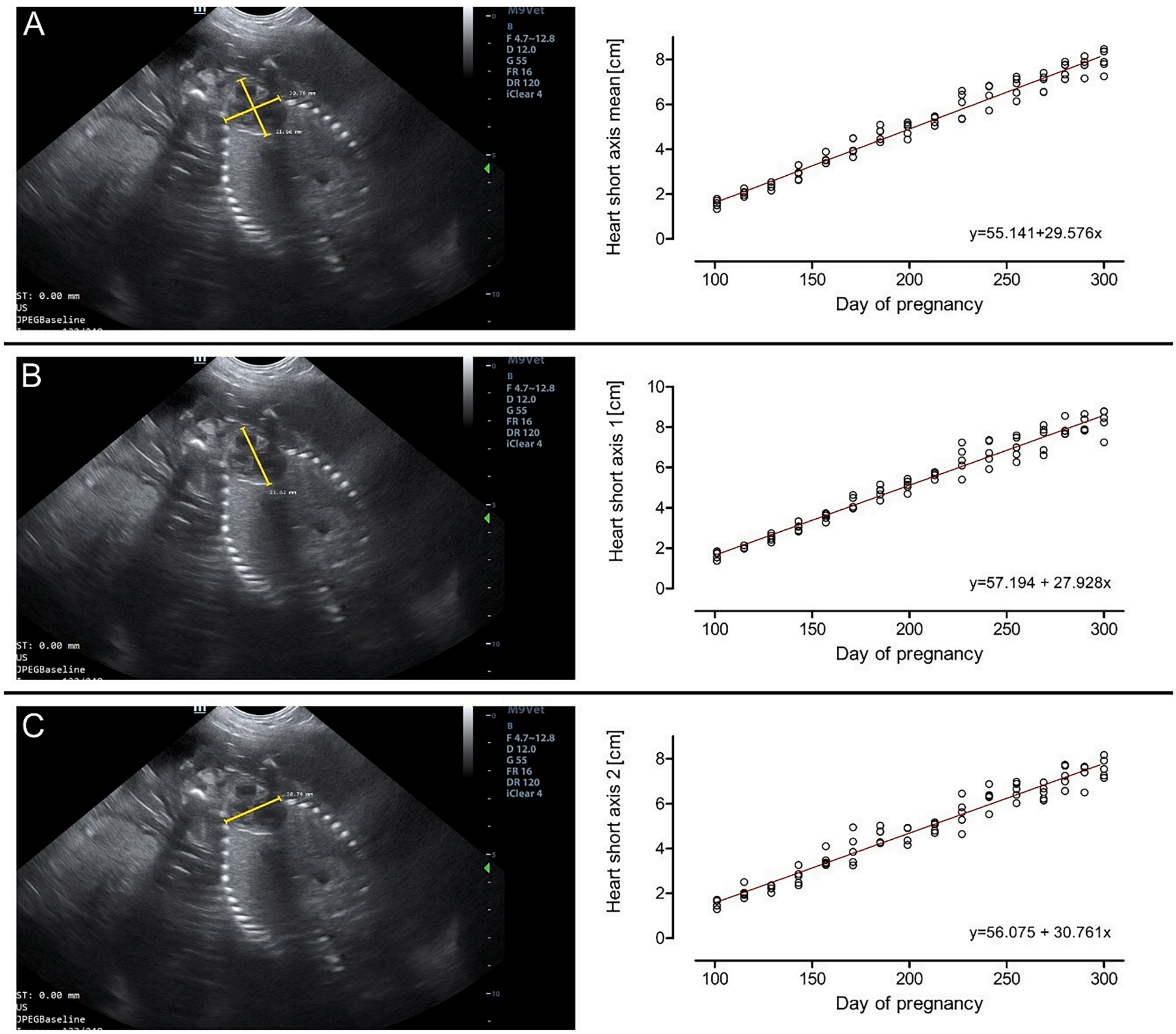

Fig. 4. Representative images for the measurement of (A) the mean fetal heart short axis (yellow line, frontal plane), (B) of the fetal heart short axis 1 (yellow line, frontal plane) and (C) the fetal heart short axis 2 (yellow line, frontal plane) together with the respective dot-plots of the individual fetal heart short axis means, 1 and 2 measured at the specific examination timepoints during pregnancy in Shetland pony mares (n=5). Linear regression line (red) and the concordant equation are given in the figure.

were not measurable and for the two perpendicular diameters heart short axis 1 and 2, 11 out of 80 values (13.75 %) were not measurable. To be able to apply a linear regression model for those variables, missing values were replaced by the mean of the values of the examination before and after the missing value. By using the linear regression model, linear equations for each assessed biometric variable for the calculation of estimated gestational age (Table 1) and estimation for the number of days before parturition (Table 2) were generated. The accuracy of the model is indicated by the standard error of the estimate in days, and the adjusted R square (Table 1 and 2). Friedmann test was used to evaluate changes in fetal activity over time. Means are given as mean ± standard deviation. A *P*-value <0.05 was considered significant.

3. Results

All pregnancies progressed until parturition without any signs of abnormalities. Duration of pregnancy ranged from 305 days to 325 days with a mean duration of 317 ± 8 days (± SD). Parturition and the neonatal adaptation period were uneventful in all animals.

3.1. Fetal biometric assessment

During retrospective evaluation of the stored video recordings, it was possible to assess all biometric parameters, except for the fetal heart parameters, without restriction for all examinations. All biometric variables increased with ongoing duration of pregnancy ($P < 0.05$), showed a strong positive correlation with gestational age (Table 1) and a strong negative correlation with remaining days until parturition (Table 2). Representative images for all assessed biometric variables and their development during pregnancy are given in Figs. 1 to 7.

Considering the reliably assessable variables, the most accurate parameters for determination of gestational age and days until parturition were CRID, aorta diameter. The least accurate morphometric variables for the estimation of gestational age and days before parturition were the three different stomach diameters. For all variables assessed, the determination of gestational age was more accurate than the estimation of days until parturition.

3.2. Fetal organ development

By the beginning of the transabdominal evaluation period (day 101),

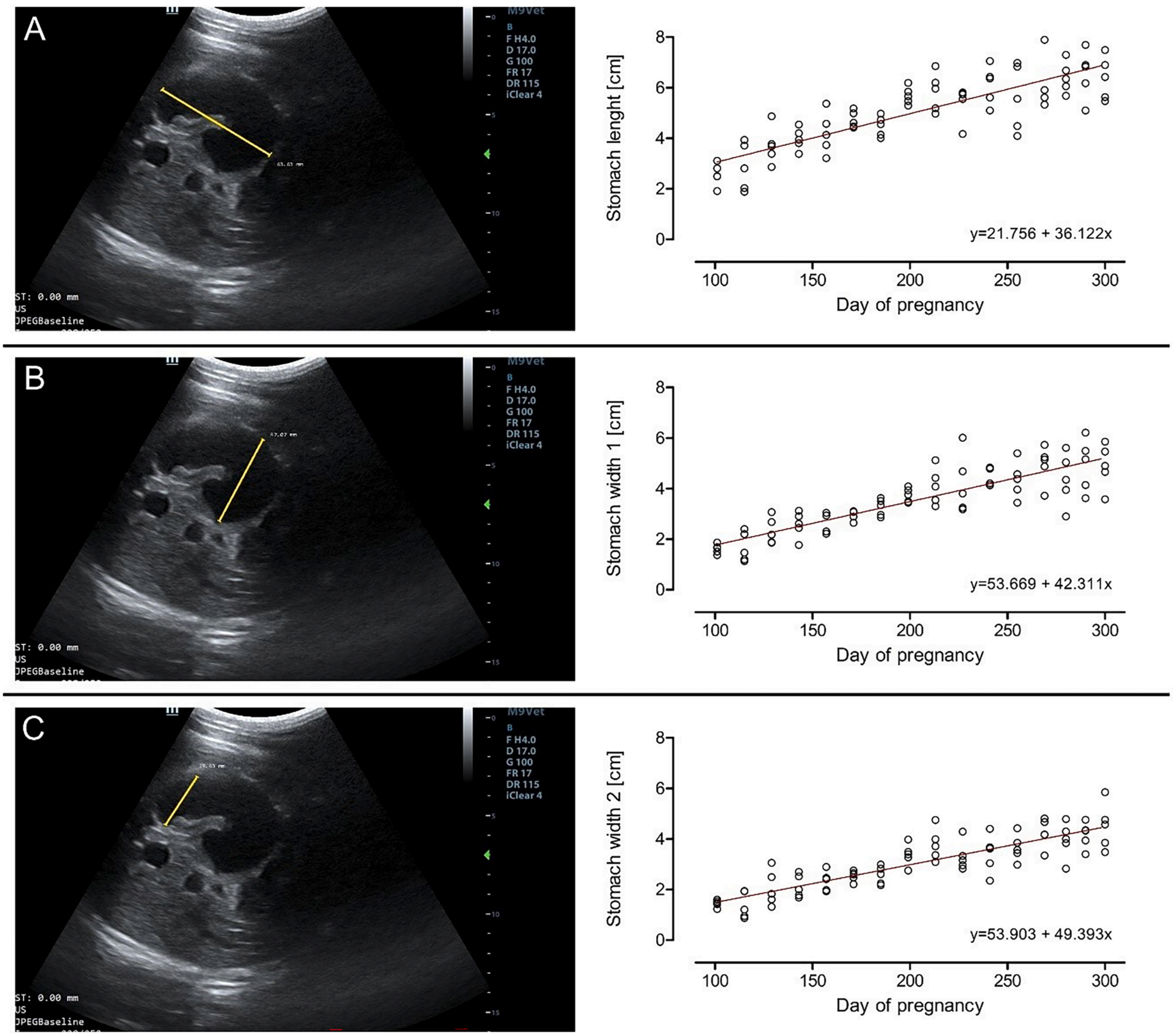

Fig. 5. Representative image of (A) the measurement of the fetal stomach length (yellow line, transversal plane), (B) fetal stomach width 1 (yellow line, transversal plane) and (C) fetal stomach width 2 (yellow line, transversal plane) together with the respective dot-plots of the individual fetal stomach length, width 1 and width 2 measured at the specific examination timepoints during pregnancy in Shetland pony mares (n=5). Linear regression line (red) and the concordant equation are given in the figure.

fetal intestines were visualizable caudal to the diaphragm in the abdominal cavity and had already reached score 1 in all pregnancies. Intestines underwent clear changes in echogenicity and ultrasonographical appearance throughout pregnancy with a continuous progression from score 2 to score 4. Score 2 was reached about midway through gestation, whereas scores 3 and 4 were only reached during the last 10 % of the total duration of pregnancy. The mean day on which the specific scores were reached are given in Table 3. Intestinal peristalsis was not reliably assessable because its determination was complicated by the permanent overlay of intestinal movements with movements of the fetus and the mare. The bean-shaped fetal stomach was detectable throughout the whole pregnancy. It showed a constant increase in size and served as an excellent landmark in the fetal abdomen to facilitate orientation. The rugae of the fetal stomach were identified by day 310 ± 10 (± SD) of gestation and around 7.0 ± 3.6 days before parturition in all pregnancies.

The developing organ characteristics and the mean day on which the different organs were detected are given in Table 4. With the applied method, a reliable differentiation between the outer gonadal border or outer border of the kidney to the surrounding tissue was not possible in the most examinations (28 of 80, 35 % and 40 of 80, 50 %, respectively) and measurements of gonadal and kidney length were therefore not included into further analysis.

The differentiation between pulmonary and hepatic tissue was possible on day 101 (n=1) or day 115 (n=4) of pregnancy. Prior to that time point, both tissues displayed similar echogenicity, but the lung showed a marked increase in echogenicity by day 115 compared to the liver. Both organs showed marked differences in echogenicity throughout pregnancy (Table 4, Fig. 7A). The fetal activity was greater during the first to second third of pregnancy, decreased around day 185 and remained reduced thereafter until parturition (Fig. 8).

4. Discussion

This study provides the first descriptive assessment of fetal organ development in Shetland ponies using a transabdominal ultrasonographic approach. The data that were analyzed retrospectively clearly suggest that biometric evaluation allows to determine gestational age and the remaining days until parturition with similar accuracy in Shetland mares as in larger horses [2,6–9,18]. Determination of the diameter of the aorta and the combined rib and intercostal distance were reliably possible throughout gestation. Considering reliably assessable

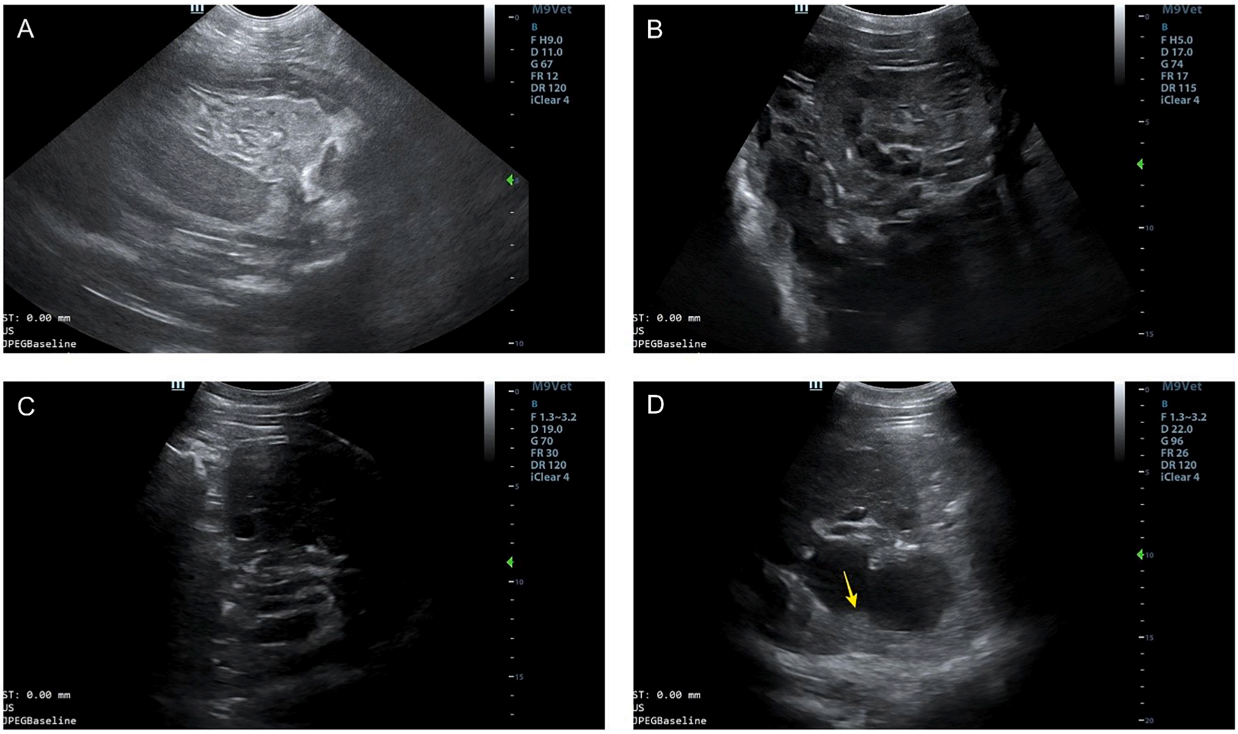

Fig. 6. Representative ultrasonographic images (transversal plane) of the scores used for assessment of fetal intestinal development: Image A = Score 2, image B = Score 3, image C = Score 4 and image D shows an equine fetal stomach with rugae indicated by yellow arrows.

Table 1
Linear regression equations defining the relationship between gestational age and the various biometric variables used for gestational age estimation between 101 days of gestation and parturition in Shetland pony mares (n=5).

Characteristic	Correlation coefficient	P-Value	Equation	Std. Error of the Estimate (± days)	Adjusted R Square	P-Value
CRID	0.977	<0.001	y=68.904 + 114.128x	13.6	0.953	<0.001
Aorta	0.968	<0.001	y=21.809 + 211.910x	15.9	0.936	<0.001
Eye diameter x	0.911	<0.001	y=-41.501 + 100.964x	26.1	0.829	<0.001
Eye diameter y	0.929	<0.001	y=-44.297 + 103.668x	23.5	0.860	<0.001
Eye mean diameter	0.927	<0.001	y=-46.799 + 103.917x	23.7	0.858	<0.001
Stomach lenght	0.835	<0.001	y=21.756 + 36.122x	34.9	0.693	<0.001
Stomach width 1	0.853	<0.001	y=53.669 + 42.311x	33.1	0.725	<0.001
Stomach width 2	0.860	<0.001	y=53.903 + 49.393x	32.4	0.736	<0.001
Heart long axis height*	0.968	<0.001	y=59.584 + 30.821x	15.8	0.937	<0.001
Heart long axis width*	0.984	<0.001	y=54.487 + 25.240x	11.3	0.968	<0.001
Heart short axis 1*	0.984	<0.001	y=57.194 + 27.928x	11.4	0.967	<0.001
Heart short axis 2*	0.977	<0.001	y=56.075 + 30.761x	13.5	0.954	<0.001
Heart short axis mean*	0.986	<0.001	y= 55.141+29.576x	10.7	0.971	<0.001

* Variables with missing values.

variables, they were also the parameters with the largest determination coefficient (adjusted R^2) for estimation of gestational age and days until parturition in the present study.

In previous publications [6–10,18], only one of the endpoints gestational age or remaining days until parturition was investigated in the same data set. Thus, the present study is the first in Shetland ponies comparing both, the accuracy of the estimation of gestational age and remaining days prior to parturition. This was possible because both, the day of ovulation and day of parturition were accurately determined for all mares. Overall, the standard error of estimate (± days) for gestational age was smaller for all biometric parameters than for the endpoint of remaining days until parturition. Whereas embryonic and fetal development follows a very precise time pattern in the horse (reviewed by Ginther [24]), pregnancy length is highly variable in this species (e.g. [25]) and final fetal maturation required for adaptation of the newborn to the extrauterine environment takes place considerably later than in other species as for example in sheep [26]. This explains why the accuracy of estimation of the days of gestation is better than the accuracy for prediction of the foaling date as could be confirmed in the present investigation.

With a high accuracy for estimation of fetal age and the remaining days until parturition as well as high coefficients of determination, CRID was the most accurate endpoint in this study. The present results do not agree with previous investigations where the authors reported a high variation in CRID because of reflectory breathing-like movements of the fetal thorax, limiting the value of this parameter for estimation of gestational age [6,9]. This might be explained by the smaller total size of the fetal thorax in Shetland pony pregnancies which result in a lesser effect of thoracic movements on the measurements. In addition, it must be considered that the more standardized measurements (always third to seventh rib) in this study compared to those by Renaudin et al. [9] and Kähn and Leidl [6] may have a major impact on the accuracy of this

Table 2
Linear regression equations defining the relationship between days before parturition and the various biometric variables used for estimation of remaining days until parturition in Shetland pony mares (n=5).

Characteristic	Correlation coefficient	P-Value	Equation	Std. Error of the Estimate (± days)	Adjusted R Square	P-Value
CRID	-0.961	<0.001	y =246.875-113.102x	17.6	0.923	<0.001
Aorta	-0.967	<0.001	y=296.338-213.239x	16.2	0.935	<0.001
Eye diameter x	-0.910	<0.001	y=359.940-101.554x	26.4	0.827	<0.001
Eye diameter y	-0.928	<0.001	y=362.922-104.345x	23.8	0.860	<0.001
Eye mean diameter	-0.926	<0.001	y= 365.355-104.560x	24.0	0.856	<0.001
Stomach lenght	-0.817	<0.001	y=292.646-35.609x	36.8	0.664	<0.001
Stomach width 1	-0.828	<0.001	y=259.948-41.363x	35.8	0.682	<0.001
Stomach width 2	-0.836	<0.001	y=259.995-48.377x	35.0	0.695	<0.001
Heart long axis height*	-0.951	<0.001	y=255.809-30.479x	19.7	0.903	<0.001
Heart long axis width*	-0.970	<0.001	y=261.386-25.050x	15.5	0.940	<0.001
Heart short axis 1*	-0.971	<0.001	y=258.966-27.769x	15.2	0.943	<0.001
Heart short axis 2*	-0.964	<0.001	y=259.874-30.543x	17.1	0.928	<0.001
Heart short axis mean*	-0.973	<0.001	y=260.909-29.389x	14.8	0.945	<0.001

* Variables with missing values.

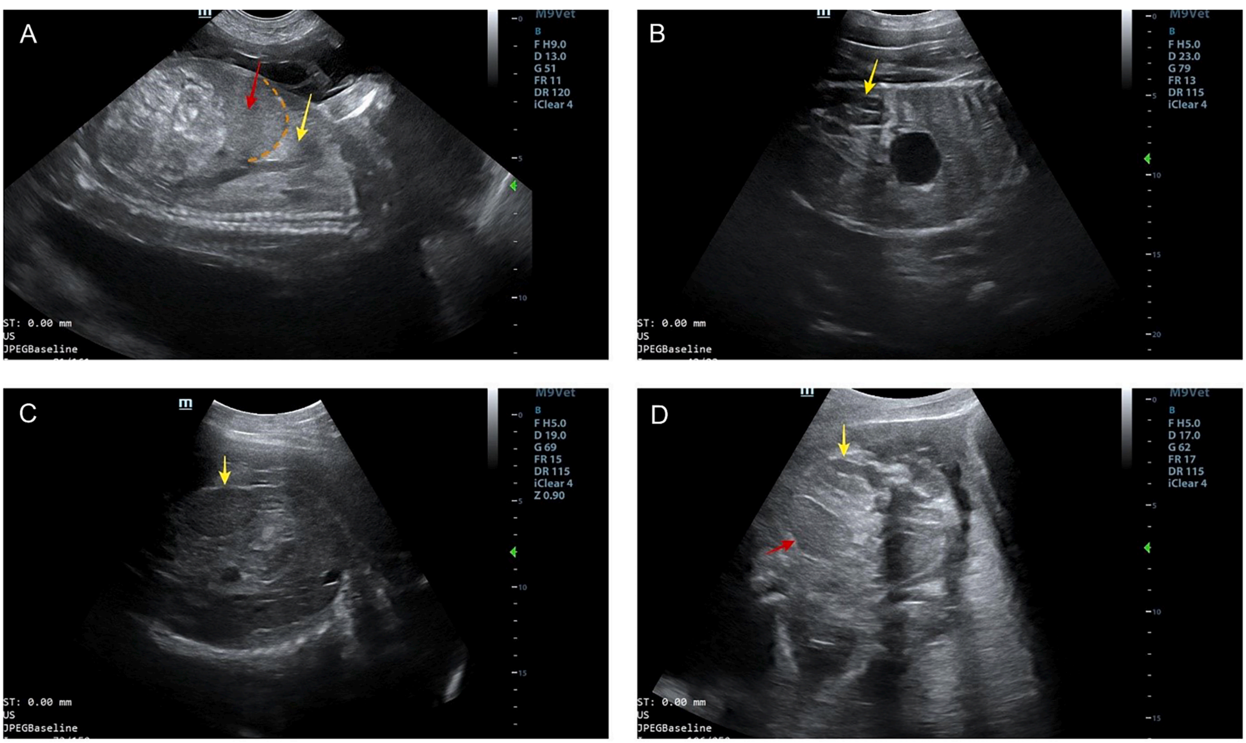

Fig. 7. Representative images of (A) the differentiation of the fetal lung (yellow arrow) and fetal liver (red arrow) tissue. The orange lines represent parts of the fetal diaphragm, (B) the fetal kidney (yellow arrow) with visible pelvic cavity (hypoechoic areas inside the renal tissue), (C) of the fetal gonads (red arrow) and (D) of the fetal gonads (red arrow) and the fetal kidney (yellow arrow). Image A and B shown in sagittal plane and image C and D in transversal plane.

Table 3
Mean day of gestation, and remaining days until parturition, at which intestinal Score 1 to 4 were reached in Shetland pony mares (n=5).

Scores	Day of Gestation	Days until parturition
Score 1	<101	> 220
Score 2	159.8 ± 6.2	157.0 ± 7.1
Score 3	285.8 ± 11.7	31.2 ± 11.6
Score 4	296.0 ± 11.4	21.0 ± 11.3

parameter. Advances in ultrasound technologies over the last 20 years do also contribute to better imaging quality in equine pregnancy monitoring [20,27].

A similar strong accuracy and high coefficient of determination was demonstrated for the diameter of the aorta, which was even more accurate than CRID for the estimation of days before parturition. The diameter of the aorta has been reported as a reliable indicator for fetal size in late pregnancy previously [8,9,16–18]. All these studies, however, indicated several factors that may impair the measurement accuracy of this endpoint. The diameter of the aorta differs between systole and diastole and should thus be evaluated consistently during systole [9]. Further, the position of measurement along the thoracal part of the aorta must be standardized as close to the heart as possible to ensure consistent measurements [16]. Both factors were accounted for and standardized in the present study. Renaudin et al. [18] further described problems in evaluation of the aorta diameter because of interference of the fetal scapula with visualization of the aorta. Similar problems with acoustic shadows produced by the scapula were not experienced in the present study, most likely because the ratio of limb length to body size in Shetland fetuses differs from that of larger horse breeds.

Table 4
Organ characteristics and day of their first detection in Shetland pony fetuses (n=5)

Organ	Characteristics	Day of first detection
Lung and liver differentiation	Change in echogenicity – lung more hyperechoic, liver tissue more hypoechoic (Fig. 7A)	Day 101 (n=1) Day 115 (n=4)
Kidneys	Elongated hypoechoic structures, in the caudal abdominal cavity cranial of the gonads, smaller in size than gonads (Fig. 7B, D)	Day 101 (n=3) Day 115 (n=2)
Renal pelvis	Hypoechoic cavity inside the renal tissue (Fig. 7B, D)	Day 115 (n=3) Day 129 (n=2)
Gonads	Oval-shaped with homogeneous echogenicity, in the caudal fetal abdominal cavity (Fig. 7C, D)	Day 101 (n=2) Day 115 (n=3)
Stomach rugae	Hyperechoic fold protruding into the anechoic lumen of the stomach (Fig. 6, D)	Day 300 (n=2) Day 310 (n=1) Day 320 (n=2)

In goats, assessment of the long and short axis of the heart were more reliable for estimation of gestational age than other endpoints [21]. To the best of our knowledge, assessment of fetal heart biometric measurements has not yet been applied to horse pregnancies. The major limitation of this parameter was the difficulty to repeat the measurements in all examinations. The relatively low standard error of the estimate as well as the high determination coefficient therefore must be interpreted with caution, as missing values were extrapolated to make the dataset usable for linear regression model analysis. It must, however, be considered that all measurements of the present study were done retrospectively on videorecorded images. Heart measurements might be more successful if done directly during ultrasonography instead.

An unexpected finding of the present study was the low determination coefficients and high deviation for all measurements determined at the fetal eye. In previous studies in Shetland ponies, eye length assessed via transrectal ultrasonography was the single best predictor of days before parturition and gestational age [10,28]. In contrast to the present study, Kähn and Leidl [6], Turner et al. [10,28] and Hendricks et al. [8] described a curve linear growth pattern of the fetal eye instead of the linear determined in the present investigation. In a more recent study [18], eye length was also no suitable parameter to estimate gestational age in riding type mares by transabdominal ultrasound. Altogether, these findings suggest differences in the growth pattern between horse breeds of different size [7,10,18,28].

The least reliable morphometric parameters were measurements of the equine fetal stomach. If the satisfactory accuracy of the previously mentioned parameters is considered, evaluation of stomach size can be dismissed for evaluation of pregnancy length in Shetland fetuses. Length measurements of the fetal kidneys and gonads were not successful in more than 40 % of the examinations which is in contrast to the situation in dogs and cats [22,29]. A possible reason might be the larger body size, responsible for difficulties with resolution complicating the differentiation between the kidney and the surrounding tissue.

In the present study, the fetal intestine was easily visualizable during every examination and structural changes according to the described scores were assessable. In contrast determination of peristalsis was not feasible in recorded videos due to the overlay of intestinal, fetal and mare movements. The given scores for intestinal development were thus only based on structural changes. Intestinal scores including peristalsis can thus not be recommended for retrospective prediction of fetal maturation which is in contrast to humans and small companion animals [22,29,30] and might even be of limited value during real-time assessment. In canine fetuses, changes in intestinal development become detectable two to three weeks before whelping [22]. In Standardbred horses, Agnew et al. [23] investigated changes of the intestine during the last four weeks before foaling. We adapted this scoring system for the present study and could detect changes in intestinal development around the transition from first to the second half of pregnancy. Further changes were detected shortly before parturition. These differences in comparison to the results presented by Agnew et al. [23] can be explained by a smaller body size and therefore shorter distance from the investigated structures to the ultrasound probe and better probe coupling because of clipping of the mares ́ ventral abdomen.

Fetal activity is a strong indicator for musculoskeletal development and reflects the function and development of the central nervous system. Under normal circumstances, fetal activity begins around day 40 of pregnancy, reaches its peak between days 60 and 100 and declines thereafter [31]. Our results agree with these findings and show a clear relation between declining fetal activity and progressing pregnancy because of fetal growth and therewith intrauterine space limitation.

5. Conclusions

To the best of our knowledge, this is the first study providing a detailed ultrasonographic assessment of fetal development in Shetland ponies. Gestational age can be estimated more accurately than days before parturition. Aorta diameter and combined rib and intercostal

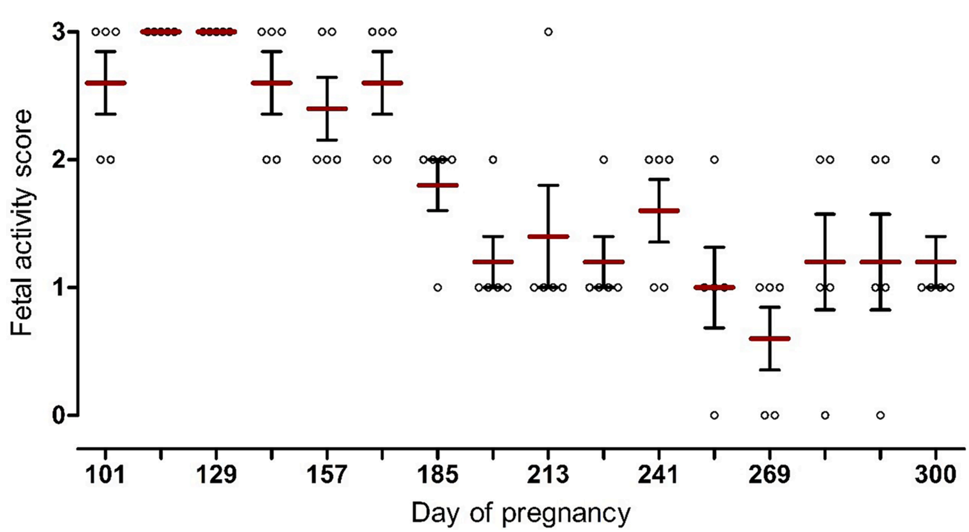

Fig. 8. Scores for fetal activity (n=5) for each examination during the evaluated period of pregnancy in Shetland ponies. Mean score per examination (red line) and standard errors of the mean (error bars) are indicated in the figure. Fetal activity decreased over time ($p \leq 0.001$; Friedman test).

distance (CRID) were the characteristics most reliably determined and providing the most accurate estimation of gestational age and days before parturition. Even in small horses like Shetland ponies, however, monitoring of development of fetal organs in the abdominal cavity is more limited than in small-sized domestic species like dogs, cats and goats. The presented results are the basis for future studies with the aim of detailed characterization of fetal biometric development of the Shetland pony fetus in a greater number of animals.

CRediT authorship contribution statement

Lisa-Hélène Wagner: Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Jörg Aurich:** Writing – original draft, Supervision, Resources, Funding acquisition. **Svenja Claaßen:** Validation, Methodology, Investigation. **Maria Melchert:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Martim Kaps:** Writing – original draft, Visualization, Validation, Methodology, Formal analysis, Data curation, Conceptualization. **Christine Aurich:** Writing – review & editing, Supervision, Software, Resources, Project administration, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors do not report any conflict of interest.

References

[1] Bergfelt DR, Adams GP. Pregnancy. In: McKinnon AO, Squires EL, Vaala WE, editors. Equine Reproduction 2nd edn. New York: Blackwell Publishing; 2011. p. 2065–79.
[2] Bucca S, Fogarty U, Collins A, Small V. Assessment of feto-placental well-being in the mare from mid-gestation to term: transrectal and transabdominal ultrasonographic features. Theriogenology 2005;64:542–57.
[3] Dascanio JJ. Assessment of late-term fetal well-being. In: Dascanio JJ, McCue PM, editors. Equine Reproductive Procedures. Iowa: John Wiley and Sons Ltd.; 2021. p. 273–6.
[4] Ginther OJ. Equine pregnancy: physical interactions between the uterus and conceptus. Proc Am Assoc Equine Prac 1998;44:73–104.
[5] Aldrich JE. Basic physics of ultrasound imaging. Crit Care Medi 2007;35:131–7.
[6] Kähn W, Leidl W. Die Ultraschall-Biometrie von Pferdefeten in utero und die sonographische Darstellung ihrer Organe. Dtsche tierarztl Wschr 1987;94: 497–540.
[7] Hartwig FP, Antunez L, Dos Santos RS, Lisboa FP, Pfeifer LFM, Nogueira CEW, da Rosa Curcio B. Determining the gestational age of crioulo mares based on a fetal ocular measure. J Equine Vet Sci 2013;33:557–60.
[8] Hendriks WK, Colenbrander B, Van der Weijden GC, Stout TAE. Maternal age and parity influence ultrasonographic measurements of fetal growth in Dutch Warmblood mares. Anim Reprod Sci 2009;115:110–23.
[9] Renaudin CD, Gillis CL, Trantal AF, Coleman DA. Evaluation of equine fetal growth from day 100 of gestation to parturition by ultrasonography. J Reprod Fertil 2000; 56:651–60.
[10] Turner RM, McDonnell SM, Feit EM, Grogan EH, Foglia R. Real-time ultrasound measure of the fetal eye (vitreous body) for prediction of parturition date in small ponies. Theriogenology 2006;66:331–7. a.
[11] Claes A, Ball BA, Brown JA, Kass PH. Evaluation of risk factors, management, and outcome associated with rectal tears in horses: 99 cases (1985–2006). J Am Vet Med Assoc 2008;233:1605–9.
[12] Eastman TG, Taylor TS, Hooper RN, Honnas CM. Treatment of rectal tears in 85 horses presented to the Texas veterinary medical center. Equine Vet 2000;12: 263–6.
[13] Sloet van Oldruitenborgh-Oosterbaan MM, Boerma S, van Exel GA, van der Holst W, Merkens HW, van Muiswinkel K, Parlevliet JM, Peters JWE, Seuren-Coppens MLG, Smeenk LAJ, van der Velden MA, van der Weijden GC. Rectal tears in the horse: malpractice or an incident? [in Dutch]. Tijdschr Diergeneeskd 2004; 129:624–7.
[14] Bucca S. Diagnosis of the compromised equine pregnancy. Vet Clin: Equine Pract 2006;22:749–61.
[15] Murase H, Endo Y, Tsuchiya T, Kotoyori Y, Shikichi M, Ito K, Sato F, Nambo Y. Ultrasonographic evaluation of equine fetal growth throughout gestation in normal mares using a convex transducer. J Vet Med Sci 2014;76:947–53.
[16] Reef VB, Vaala WE, Worth LT, Spencer PA, Hammett B. Ultrasonographic evaluation of the fetus and intrauterine environment in healthy mares during late gestation. Vet Radiol Ultrasound 1995;36:533–41.
[17] Reef VB, Vaala WE, Ll Worth, Sertich PL, Spencer PA. Ultrasonographic assessment of fetal well-being during late gestation: development of an equine biophysical profile. Equine Vet J 1996;28:200–8.
[18] Renaudin CD, Kass PH, Bruyas JF. Prediction of gestational age based on foetal ultrasonographic biometric measurements in light breed horses. Reprod Dom Anim 2022;57:743–53.
[19] Gao Y, Hannan MA, Murata K, Rajabi-Toustani R, Nambo Y. Ultrasonographic examination of equine fetal growth parameters throughout gestation in pony for Equine-Assisted Therapy. J Vet Med Sci 2022;84:74–81.
[20] Lotti F, Bertolotto M, Maggi M. Historical trends for the standards in scrotal ultrasonography: What was, what is and what will be normal. Andrology 2021;9: 1331–55.
[21] Lee Y, Lee O, Cho J, Shin H, Choi Y, Shim Y, Choi W, Shin H, Lee D, Lee D, Shin S. Ultrasonic measurement of fetal parameters for estimation of gestational age in Korean black goats. J Vet Med Sci 2005;67:497–502.
[22] Gil EM, Garcia DA, Froes TR. In utero development of the fetal intestine: Sonographic evaluation and correlation with gestational age and fetal maturity in dogs. Theriogenology 2015;84:681–6.
[23] Agnew ME, Slack JA, Stefanovski D, Linton JK, Sertich PL. Sonographic appearance of the late gestation equine fetal intestine. J Equine Vet Sci 2018;66:235.
[24] Ginther OJ. Embryology and Placentation. In: Ginther OJ, editor. Reproductive biology of the mare: basic and applied aspects. 2nd ed. WI: Equiservices Publishing; 1992. p. 345–418.
[25] Kuhl J, Stock KF, Wulf M, Aurich C. Maternal lineage of warmblood mares contributes to variation of gestation length and bias of foal sex ratio. PLoS One 2015;10:e0139358.
[26] Fowden AL, Silver M. The effects of thyroid hormones on oxygen and glucose metabolism in the sheep fetus during late gestation. Physiol J 1995;482:203–13.
[27] Ferris RA, Lu KG, Kidd JA, Lu KG, Frazer ML. Use of ultrasonography for evaluation of mare reproductive abnormalities. Atlas of equine ultrasonography, 2nd. Oxford, UK: John Wiley and Sons Ldt.; 2022. p. 331–46.
[28] Turner RM, McDonnell SM, Feit EM, Grogan EH, Foglia R. How to determine gestational age of an equine pregnancy in the field using transrectal ultrasonographic measurement of the fetal eye. Proc Am Assoc Equine Pract 2006; 52:250–5. b.
[29] Lopate C. Gestational Aging and Determination of Parturition Date in the Bitch and Queen Using Ultrasonography and Radiography. Vet Clin N Am 2018;48:617–38.
[30] Ziliantia M, Fernandez S. Correlation of ultrasonic images of fetal intestine with gestational age and fetal maturity. Obstet Gynecol 1983;62:569–73.
[31] Ginther OJ. Physical activities and morphologic aspects of the equine fetus during days 40–150. J Equine Vet Sci 2022;112:103891.