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Real-time ultrasound measure of the fetal eye (vitreous body) for prediction of parturition date in small ponies

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Abstract

Ultrasonographic fetal eye measures have been used to estimate gestational age of the fetus in light horse mares. However, fetal eye measures have not been published for smaller pony breeds. This study was conducted to develop reference ranges for ultrasonographic measures of fetal eyes in small ponies for the purpose of predicting days before parturition (DBP) when breeding or ovulation dates are unknown. Twenty-three Shetland-type pony mares were studied across one ($n = 10$) or two ($n = 13$) gestations in 2004 (18 pregnancies) and 2005 (18 pregnancies). Measurements of fetal eyes were obtained during transrectal ultrasound examination. Examinations were conducted once monthly in a field situation beginning in December (2003) or August (2004) until mares foaled (March through July). The length (from sclera to sclera) and width (from retina to cornea) of the vitreous body were measured. For the 273 examinations in which gestation age was greater than 2 months, eye measures were obtainable in 248 (91%). Mixed-effects linear regression modeling was used to account for serial growth measures within pregnancy, repeated measurements across mares, and unbalanced study design. Independent variables evaluated included vitreous body length, vitreous body width, the ratio of length to width, parity, and mare height at the withers at parturition. Eye length was the best single predictor of days before parturition, with almost no additional predictive value of the other variables considered. Our resulting regression equation is: days before parturition = 265.16 − 0.21*(vitreous body length in mm)^2. This study suggests that measure of the fetal eye is a practical on-farm procedure for estimating days before parturition in small ponies.

Keywords: Pony; Mare; Fetus; Eye; Parturition; Gestation; Ultrasonography

1. Introduction

Ultrasonographic measures of the fetal eye have been used to estimate gestational age of the fetus in horses, cattle, sheep, fallow deer, and humans [1–5]. In horses, normal ranges for fetal eye measures at different stages of gestation have been reported for light horse breeds, but not for small pony breeds such as Shetland, Dartmoor, and Welsh. Since small pony foals are considerably smaller than light horse foals, it is generally presumed that fetal eye measures for light horse fetuses are proportionately larger than measures from age-matched small pony fetuses and therefore would not accurately predict gestational ages of small

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pony fetuses. The present study was conducted to develop reference ranges for transrectal ultrasonographic measures of fetal eyes to predict days before parturition (DBP) in small ponies. Our specific objectives were to measure fetal eyes in small ponies across gestation, to calculate growth curves, and to develop practical reference ranges for predicting days before parturition when breeding or ovulation dates are unknown.

2. Methods

2.1. Subjects

Twenty-three pony mares, ranging in age from 1 to 15 years, were studied across the gestation of their 2004 and 2005 foals. These Shetland-type (100–250 kg) mares are maintained together with stallions in a semi-feral pony herd at the University of Pennsylvania, New Bolton Center, Chester County, Pennsylvania, for the study of reproductive physiology and behavior under semi-natural conditions. The herd has been maintained since 1994 under natural social and breeding conditions, with minimal human contact and management. No breeding management or reproduction interventions or examinations are done as a matter of course in this herd. Behavior observations vary in intensity from year to year, depending upon observation schedules for other ongoing behavior research. During the course of this study, with few exceptions, breeding dates were not systematically known.

This semi-feral herd is maintained within an enclosure of approximately 50 acres that includes natural grass pasture, light forest with browse, three streams, a pond, and seasonal bogs. The herd maintains good body condition foraging on natural grasses and browse for most of the year. Supplemental native grass hay is distributed when natural forages are depleted in deep winter.

Eighteen pregnancies were studied in each year (36 total pregnancies). Thirteen of the 18 mares from the first year were included in the second year. Nine of the pregnancies were primiparous in yearling fillies that had conceived between 11 and 16 months of age (mean 13.5, S.D. 1.6 months), and 27 were multiparous (2nd–10th gestation). Monthly examinations were conducted on study mares from December 2003 until parturition in April through July 2004 and from August 2004 until parturition in March through July 2005.

Parturitions were largely unattended and, based on observation every 4 h when foals are expected, typically occurred just before dawn. All foals appeared to be full term at birth and were healthy with the exception of one foal from a primiparous mare that was extracted dead following a dystocia. No adverse effects of repeated ultrasound examinations were evident.

2.2. Ultrasonographic examinations

Transrectal ultrasonographic examinations of the fetuses were performed under field conditions. Before examination, each harem group was contained within a 13 m $\times$ 26 m catch pen. Each study mare was haltered and led into a chute within the catch pen. Mares were restrained in cross-ties and transrectal examinations were performed using portable ultrasound units equipped with 7.5 mHz linear array transducers (Sonovet 600 and Sonovet 2000 portable ultrasound machines, Universal Medical, Bedford, NY). Ultrasound units were positioned within opaque cases to decrease sun glare.

From December 2003 through December 2004, two veterinary clinicians participated in each monthly session, each performing approximately half of the examinations. For the remainder of the study, one of those clinicians conducted all examinations. Clinicians remained blind to any known breeding dates, previous parturition dates, or results of previous monthly examinations. If the fetal eye could not be located by the first examining clinician, then, when a second clinician was available, that clinician also examined the mare. Only in rare cases was the second clinician able to locate an eye following an initially unsuccessful examination by the first clinician.

Transrectal examinations were performed in a routine manner. Briefly, for each examination, the rectum was evacuated of feces and the mare's uterus and cervix were examined by palpation per rectum. The ultrasound transducer then was introduced into the rectum and the uterine contents were examined. The transducer was swept from left to right over the uterus starting at the internal cervical os and gradually proceeding cranially until an orbit was identified. The transducer was passed over the eye to image the longest (from medial sclera to lateral sclera) and widest (from retina to cornea) dimensions of the vitreous body. This image was frozen and measurements of the length and width of the vitreous body were obtained. To standardize the plane of beam emission, the lens was included in the frozen image and the vitreous width measured with the cursor line bisecting the lens at a 90° angle and then the length measured perpendicular to the width line (Fig. 1).

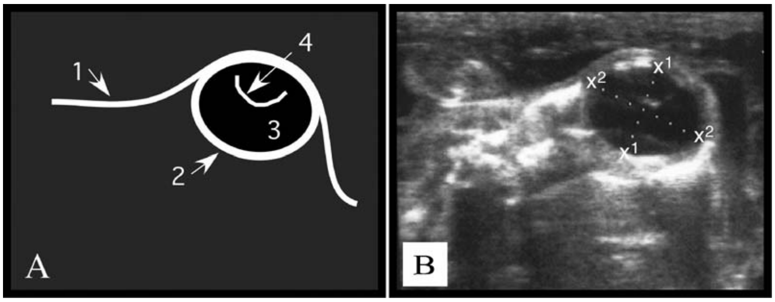

Fig. 1. Schematic representation and actual ultrasonogram of a fetal eye. In the schematic (A), the numbers indicate various parts of the fetus depicted in the actual image. (1) Skull; (2) bony orbit; (3) vitreous; (4) lens. In the actual image (B), the cursors are delineating the width (1) and length (2) of the eye. Note that the width cursor is bisecting the fetal lens at an approximately 90° angle. The length cursor is perpendicular to the width cursor.

After the measures were recorded, the image was unfrozen and the scan and eye measurements were repeated twice to obtain a total of three sets of length and width measures. If measures were not obtained, the clinician's explanatory notes were recorded.

2.3. Other measures

In both years, date of birth, gender of each foal, height of the mare at parturition, and body condition score (BCS) of each mare in March (winter low) were recorded. In 2005 only, the weights of all foals ($n = 18$) were obtained within 8 h of parturition.

2.4. Statistical analysis

For each examination, a mean of each of the sets of three measures of length and width were calculated. Following parturition, we calculated the days before parturition on which each examination was performed. Using DBP as the dependent variable, mixed-effects linear regression modeling procedures were employed to account for serial growth measures within pregnancy, repeated measurements across mares and unbalanced study design, using the nlme package in the R statistical language (R v. 2.010 with nlme v. 3.1–65, The R Foundation, 3.1–65, Vienna, Austria, www.r-project.org) [6]. Independent variables evaluated included vitreous body length, vitreous body width, the ratio of length to width, parity, and mare height at the withers at parturition. To account for curvature in the growth pattern, several forms of regression equation were considered including second degree polynomials and logarithmic forms. The model was validated using a jackknifing procedure.

3. Results

3.1. Success of obtaining eye measures

For 2004 foals, 3–7 monthly examinations were conducted per pregnancy. For 2005 foals, 8–11 monthly examinations were conducted per pregnancy. These resulted in three to nine successful eye measures for each pregnancy. Although we attempted to obtain eye measures regardless of gestational age, we were not successful in any pregnancies determined by ultrasonographic appearance to be less than 2 months of gestation. This is consistent with previous reports describing the appearance of the equine conceptus during the first 50 days of gestation [7,8].

At the end of the study, fetal age for each examination was estimated using information recorded

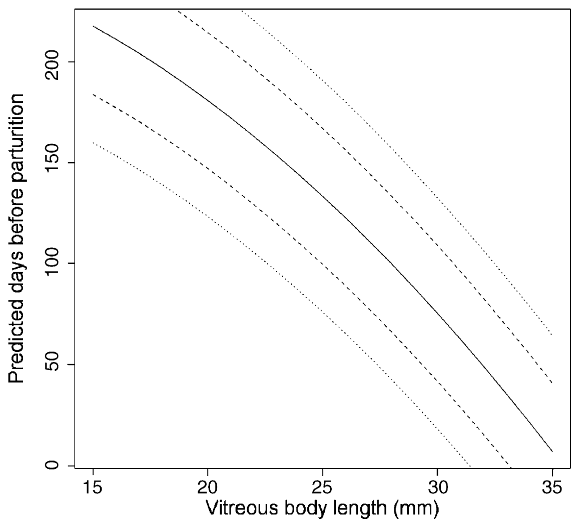

Fig. 2. Graphic depiction of regression curve showing 75 and 95% prediction intervals for medial to lateral vitreous body length (mm).

on palpable size of the uterus, ultrasonographic appearance of the early conceptus, any breeding observations, and the date of parturition. For the 273 examinations in which fetal age was estimated as in the third month or greater of gestation, an eye was successfully measured in 248 (91%) examinations. Failures were most frequent during months 3 (35% of examinations) and 4 (19% of examinations) of gestation, least frequent during months 8 (2.5%), 9 (0%), 10 (8%), and 11 (0%), and intermediate during months 5 (12%), 6 (12%), and 7 (13%). In early pregnancies, failures were attributed to low position of the fetal head and/or posterior presentation of the fetus at the time of the examination. During later gestation, failures were attributed to low position of the fetal head at the time of examination. Within individual pregnancies, failures to obtain eye measures ranged from 0 to 40% of examinations.

3.2. Regression model

The mixed-effects linear regression resulted in a regression equation of DBP = 265.16 − 0.21*(vitreous body length in mm)^2. The mixed-effects procedure also estimates the variation in growth curves across mares and across pregnancies within each mare. The data indicate significant variation in the growth pattern across mares and little variation across pregnancies within the same mare, at least in the 2 years studied (Table 1). The residual standard error for the model is 29.06 indicating that, within an individual pregnancy, measured length tends to vary around the smooth growth curve. This may indicate a sporadic growth process or noise in the ultrasound measurements. Table 2 lists prediction intervals for eye lengths from 15 to 35 mm based on this model. Fig. 2 depicts the regression curve for the data.

Several alternative models were considered. Additional possible explanatory variables evaluated but which entered without a significant coefficient ($P > 0.1$) were: unsquared eye length, eye width, the eye length times the eye width, mare height, parity, and foal gender. Accordingly, these were not included in the model.

Table 1
Coefficients of mixed-effects linear regression model relating days before parturition (DBP) with the square of the vitreous body length (mm)

	Population mean effect	S.D. across mares	S.D. across pregnancy within mare
Regression Coefficient (days-to-parturition = $A - B$*(length)			
A (days)	265.16	13.88	0.18
B (days/mm^2)	−0.21	0.01	0.00

Apparent in our raw data was the tendency for elongation of the eye from round to oval as gestation advanced. When ratio of length to width is added to the model, it is a significant additional predictor ($P < 0.01$). While the change in ratio is statistically significant, it has relatively low explanatory power and only reduces the residual standard error from 29.06 to 28.16 days. Considering the increased effort of obtaining width measures, we concluded that the benefit of adding length-to-width ratio was not warranted, particularly in a field situation.

The final model was validated using a jackknifing procedure. For each mare, we removed that mare's data points (from all gestations) from the dataset and re-estimated the model. The new model was used to predict the DBP for each length measurement that was taken for the hold-out mare. We then calculated the prediction error between the new model and the actual DBP for each measurement. This procedure was repeated for each mare and the mean absolute prediction error across mare is 25.97 days. This indicates the capability of the model to predict DBP for other small pony mares.

3.3. Comparison to light horse mare measures

A premise of this study was that small pony fetuses would have smaller eye measures, and possibly different growth curve characteristics than those previously

Table 2
Prediction mean with 75 and 95% prediction interval ranges for days before parturition

Eye length (vitreous body medial to lateral, mm)	Days before parturition		
	Mean	75% PI	95% PI
15	209	184–251	160–275
16	203	177–245	153–269
17	197	170–238	146–262
18	190	153–230	140–254
19	183	155–223	131–238
20	175	147–214	123–238
21	167	138–206	115–230
22	158	129–197	106–220
23	150	120–187	96–211
24	140	110–177	86–201
25	131	100–167	76–191
26	121	89–156	65–180
27	110	78–145	54–169
28	99	66–133	43–157
29	88	54–121	31–145
30	77	42–109	18–133
31	65	29–96	5–120
32	52	16–83	0–107
33	39	2–69	0–93
34	26	0–55	0–79
35	13	0–41	0–64

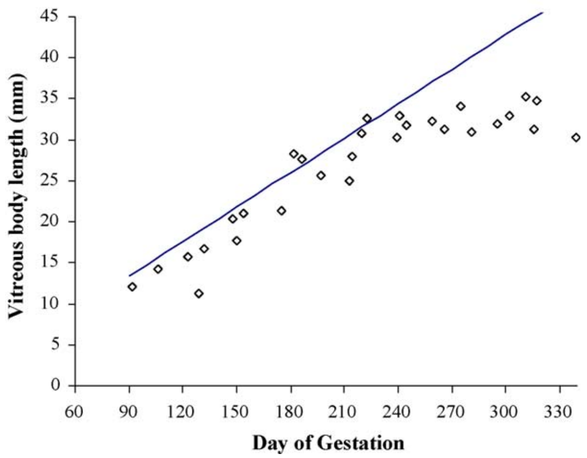

Fig. 3. Sub-set of eye measures from four pony fetuses of known gestational age plotted against regression line of Kahn and Leidl [1] for fetuses of light horse mares.

published for light horse mares. Since DBP was the dependent variable in our model, direct comparisons of our results with published data for light horse mares which used days of gestation as the dependent variable were not possible. For four fetuses for which we could establish gestational age based on ultrasonic appearance of the conceptus before 60 days gestation [7,8], we were able to compare our eye measures of pony festuses to those of Kahn and Leidl for light horse mares. For each of the four fetuses for which gestational age was established, 7 or 8 monthly examinations were performed for a total of 29 sets of eye measures. Fig. 3 depicts those eye lengths in relation to the regression line reported by Kahn and Leidl for light horses [1]. Twenty-six of our 29 pony eye lengths fall below the regression line, indicating that pony fetal eye measures are in fact smaller than those of light horse mares. Since measures of dispersion were not reported in the Kahn and Leidl regression model, we are not able to directly compare growth curves statistically. Visual inspection indicates that the eye growth pattern of these four fetuses was linear and parallel to that of light horse mares in early pregnancy, but plateaued beginning about month 9 of gestation.

3.4. Influences on eye growth and size at parturition

A number of possible influences on fetal eye measures were evaluated.

3.4.1. Year of parturition

Eye length and width obtained at ultrasonographic examinations conducted within 30 days of parturition were similar among the foals born in 2004 ($n = 18$) and 2005 ($n = 18$) (independent t-test, 34 d.f., $P > 0.10$).

3.4.2. Foal gender

Male (length 31.7 mm; width 26.5 mm; $n = 18$) and female (length 32.2 mm; width 27.0 mm; $n = 18$) eye measures within 1 month before parturition were similar (independent t-test, 34 d.f., $P > 0.05$).

3.4.3. Parity

The primiparous mares in this study were all 2-year-old fillies that had not yet reached mature heights and weights. Birth weights of foals of the four 2-year-old primiparous mares (2005) averaged 14.5 kg while birth weights of foals of the multiparous mares (ages 3–15 years) averaged 22.5 kg. This difference in birth weight is highly significant (independent t-test, 16 d.f., $P < 0.01$). However, both eye length and width of fetuses of primiparous and multiparous mares within the last month before parturition were similar (independent t-test, 34 d.f., $P > 0.10$). For the 18 foals of 2005, the association of birth weight and eye length ($r = -0.10$, $P > 0.10$) and width ($r = -0.25$, $P > 0.10$) within 1 month before parturition were not significant.

3.4.4. Body condition

Body conditions among ponies of this herd are lowest in late winter. For the mares in this study, BCS for March 2004 and March 2005 ranged from 3 to 7.5, with an average of 6.2 on the Henneke scale (www.draftresource.com/draft_body_condition.html). For 2005, where newborn foal weights were available, foal weights for mares with BCS less than 6 ($n = 3$) were significantly lower than those for mares with scores 6 and higher ($n = 15$; independent t-test, $P < 0.05$). However, fetal eye length at the examination closest to parturition (2–36 days before parturition in 2005) for those mares of lower and higher March BCS were not significantly different (independent t-test, $P > 0.1$). Considering data from both years (36 pregnancies), fetal eye length at the examination closest to parturition was also not significantly associated with mare BCS in March (Pearson $r = 0.11$).

3.4.5. Size of mare

Height of mares at the withers at the time of parturition ranged from 41 to 46 in. (43.9 in.). Considering data from both years (36 pregnancies), fetal eye length at the examination within 1 month of parturition was not significantly associated with mare height (Pearson $r = -0.11$).

4. Discussion

The goal of this study was to develop reference values for transrectal ultrasonographic measures of fetal eyes in small ponies. We used data obtained in monthly measures of 36 gestations over a 2-year period to generate a regression model using vitreous body length to predict DBP. A model using the square of the vitreous body length proved to be the best fit for eye measures and DBP. Of eye width and length measures, length was the better single predictor of DBP. Although width also was a significant single predictor of DBP, it was so highly correlated with length that it did not provide significant additional predictive value.

Our regression analysis yielded a curvilinear model of growth for fetal eye length in these pony fetuses. This is in contrast to a linear model reported for light horse fetuses and fetuses of other species [3–5]. The majority of the Kahn and Leidl light horse fetal measures were taken in mid gestation. Those authors reported that the fit to their linear model is fairly close in early and mid gestation, but not in later gestation, where only 6 of their 96 observations contributed to the model after day 240 of gestation and none after day 280. Further data with light horses would be required to determine whether there truly is a difference in the growth curves of pony and light horse fetal eyes. Plot of a sub-set of our pony data for which gestational age was known against the regression line published for light horse fetuses confirms the premise of this study that actual size of pony fetal eyes is smaller.

Transrectal ultrasonographic measurement of the equine fetal eye is a straightforward on-farm procedure for the veterinarian experienced with transrectal evaluation of the mare's reproductive tract. Our study was done under field conditions with semi-feral mares. Yet even under these conditions, we experienced minimal difficulties when performing this procedure and were able to locate the eye and obtain measures in over 90% of examination attempts beyond 2 months of gestation.

In this study, at each examination we obtained three separate measures of vitreous body length and width from which mean length and width were calculated as our ‘‘best estimate’’ for each eye dimension. Within these sets of three measures at each examination, disparity ranged from 0 to 8 mm (mean 2.02 mm; S.E. 0.08 mm; $n = 224$) for eye length and 0–8 mm (mean 1.94 mm, S.E. 0.09 mm; $n = 224$) for eye width. Judging that this disparity within sets of repeated measures was fairly low, we considered the benefit of the additional effort of three measures over a single measure. We re-fitted our resulting model using only the first of the three length measures. The residual error of the single-measure model was 32.98 days versus 29.06 days for the three-measures model. The mean absolute prediction error for the jackknife validation was also slightly larger (26.15 days versus 25.97 days). Accordingly, we decided that the predictive value gained by using the mean of three-measures outweighs the minimal additional effort of taking two additional measures. If, however, only one or two measures of length were obtainable, our data would support the recommendation to use our three-measures model, expecting the prediction interval to be somewhat broader.

The design of this study precluded evaluation of differences due to ultrasonographic technique (i.e. operator, equipment) since there was systematic assignment of smaller, primiparous mares to one clinician and larger, multiparous mares to the other clinician, and since one clinician consistently used one particular ultrasound machine while the other clinician switched machines over the course of this study. Further work designed to address operator and machine variation would be necessary to evaluate these factors.

Our study design differed from previous studies in which fetal eye measures were obtained on a known day of gestation based on last breeding date to formulate a model for predicting gestational age of the fetus [1]. Since, in our study sample, ovulation or last breeding dates were known with certainty in only four instances, we used the date of parturition to retrospectively calculate the DBP on which each set of eye measures had been obtained. The resulting prediction intervals are wider compared to those of a previous study of light horse mares in which fetal eye measurers were determined for fetuses of known gestational age [1]. Our prediction intervals reflect variation due to both development of the foal and to the natural wide variation in mare gestation length. Gestation length in the mare has been reported to range from 310 to 374 days [9,10]. While on the surface it may appear that the wide intervals in predicting DPB would be less practical than predicting day of gestation with a narrower prediction interval, the practical value of the two approaches are likely similar. Predicting DBP explicitly accounts for the natural variation in gestation length, whereas predicting day of gestation does not.

Although foals from primiparous pony mares in this study weighed significantly less than foals from multiparous pony mares, eye measures did not differ significantly between the two groups. The disparity in foal birth weight is expected since mares of this herd begin ovulating and become pregnant in their yearling breeding season, often before 1 year of age. Therefore, these primiparous mares have usually not reached their mature physical size until after their second foal is born when they are 3 years old. Additionally, fetal eye measures did not appear to be affected by the mare's BCS near parturition or the mare's height, even though newborn foal weight was lower with shorter mares and mares with lower BCS. These data indicate that eye size near parturition does not vary directly with size of foal within this limited size range and suggests that variations in fetal size within a breed might not be reflected in fetal eye measures. Although we did not address breed differences in this study, the question arises as to whether these results would be useful for predicting gestation length of fetuses from non-Shetland-type breeds of similar sizes. Although we did not see variation in fetal eye size near birth within the range of sizes in our group of small Shetland-type ponies, it is reasonable to expect that ratio of eye size to skull width may vary as a characteristic of breed, and may confound application of prediction models among breeds, even among breeds of similar size.

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References

[1] Kahn VW, Leidl W. Die ultraschall-biometrie von pferdefeten in utero und die sonographische darstellung ihrer organe. Dtsch tierarztl Wschr 1987;94:497–540.

[2] Kahn W, Kahn B, Richter A, Schulz J, Wolf M. Sonography during the pregnancy of sheep. I. Fetometry for the determination of the stage of gestation and prediction of the time of parturition. Dtsch tierarztl Wschr 1992;99:449–52.

[3] Lenz MF, English AW, Dradjat A. Real-time ultrasonography for pregnancy diagnosis and foetal ageing in fallow deer. Aust Vet J 1993;70:373–5.

[4] Revol B, Wilson PR. Ultrasonography of the reproductive tract and early pregnancy in red deer. Vet Rec 1991;128:229–33.

[5] Goldstein I, Tamir A, Zimmer EZ, Itskovitz-Eldor J. Growth of the fetal orbit and lens in normal pregnancies. Ultrasound Obstet Gynecol 1998;12:175–9.

[6] Laird NM, Ware JH. Random-effects models for longitudinal data. Biometrics 1982;38:963–74.

[7] Ginther OJ. Ultrasonic imaging and reproductive events in the mare. Cross Plains: Equiservices; 1994. p. 378.

[8] Kahn W. Veterinary reproductive ultrasonography. Hannover: Mosby-Wolfe; 1994. p. 256.

[9] Howell C, Rollins W. Environmental sources of variations in gestation length of the horse. J Anim Sci 1951;10: 789–96.

[10] Rossdale PD, Ricketts SW. The practice of equine stud medicine. Baltimore: Williams and Wilkins; 1974.