How to Determine Gestational Age of an Equine Pregnancy in the Field Using Transrectal Ultrasonographic Measurement of the Fetal Eye

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1. Introduction

In equine practice, pregnant mares may be presented for examinations without known breeding or ovulation dates. In these instances, owners frequently ask the veterinarian to estimate the age of the pregnancy or the parturition date. Depending on the stage of gestation, this can be a difficult task, and practitioners would benefit from a straightforward field method for accurately determining gestational age of a mare's pregnancy. In early pregnancy (before approximately day 70), the gestational age of the pregnancy can be reliably determined using transrectal palpation and transrectal ultrasonographic examination of the conceptus. During this time, the characteristic palpable size and ultrasonographic size and appearance of the conceptus allow the practitioner to rapidly and accurately estimate gestational age.[1] Beyond ~day 70, the conceptus becomes too large and descends too far over the pelvic brim to be reliably examined in its entirety by transrectal approaches. Thus, in mid to late gestation, transabdominal ultrasonographic examination of the conceptus has been used to obtain multiple measurements (e.g., size of the fetal eye, intercostal distance, skull diameter, and heart rate) that can be used to estimate gestational age.[2] Unfortunately, transabdominal ultrasonographic examination of the conceptus is time consuming. Additionally, it requires specialized ultrasonographic equipment that is often not available to the general equine field practitioner, and it requires clipping of a large portion of the ventral abdomen (a procedure that is usually unappealing to the horse owner). A measurement that could be obtained per rectum and that enables accurate estimation of gestation length or days before parturition would be more desirable to most practicing veterinarians.

NOTES

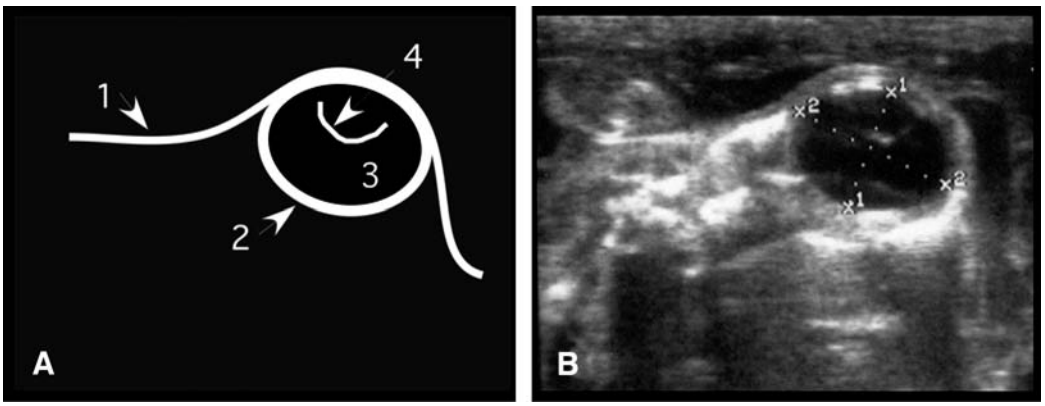

Fig. 1. (A) Schematic representation. The numbers indicate various parts of the fetus depicted in the actual image: (1) skull, (2) bony orbit, (3) vitreous, (4) lens. (B) Actual ultrasonogram of a fetal eye. The cursors are delineating (1) the width and (2) the length of the eye. The lens is included in the image and helps to insure that measurements are standardized. Reprinted from *Theriogenology*, Vol 66, Turner, R.M., McDonnell, S.M., Feit, E.M., Grogan, E.H. and Foglia, R.F., Real-time ultrasound measure of the fetal eye (vitreous body) for prediction of parturition date in small ponies, 331–337, Copyright (2006), with permission from Elsevier.

Out of the above-described group of transabdominal measures, measurement of the size of the fetal eye is the one parameter that generally can be readily and reliably obtained using transrectal ultrasonography, even during mid to late gestation.[3] Transrectal ultrasonography, unlike transabdominal ultrasonography, is a technique that is time efficient and familiar to most equine veterinarians; additionally, this technique is one that requires only standard ultrasonographic equipment that is available to many field practitioners. No clipping of hair is required. The objective of this abstract and presentation is to introduce new information on how to best estimate gestation length or days before parturition in mid- to late-gestation horse and pony mares using only transrectal ultrasonographic measurement of the fetal eye.[4]

2. Materials and Methods

Preparation of the Mare

The mare to be examined should be safely restrained for routine examination per rectum. For most tractable mares, restraint may consist of only a halter and lead shank held by a competent handler or a halter and cross ties. Ideally, the examination should be conducted in a chute or stocks to restrict lateral movement of the mare during the examination. For less tractable mares, a twitch can be included or the mare can be sedated with a low dose of xylazine, detomidine, acepromazine, or some combination of these drugs. In our study of pregnant semi-feral pony mares, animals were crosstied in a chute for examination, and only 1 of 23 mares required additional restraint in the form of a twitch and/or sedation before a safe examination could be performed. Many of these mares were maiden mares that had no previous exposure to examination per rectum, but the vast majority still tolerated the procedure well.

Examination of the Fetal Eye

After the mare is restrained as described above, the clinician should prepare for standard transrectal examination of the uterus by donning a clean palpation sleeve and applying appropriate amounts of lubricant to the sleeved arm. The mare's rectum is then emptied of feces, and the uterus is located and examined manually for tone, size, and shape. To obtain measurements of the fetal eye, the ultrasound transducer[a] is introduced into the rectum, and the uterine contents are examined. The transducer is swept from left to right over the uterus starting at the internal cervical os and gradually proceeding cranially until an orbit is identified. The fetal orbit is very characteristic in appearance and can be readily identified ultrasonographically. The sagittal section of the anechoic fetal eye within the orbit is roughly circular in shape during early to mid gestation, and it becomes more oval during mid to late gestation (Fig. 1). The transducer is passed over the eye to image the longest (from medial sclera to lateral sclera) dimension of the vitreous body. This image is frozen, and a measurement of the length of the vitreous body is obtained. To standardize the plane of beam emission, the lens should ideally be included in the frozen image (Fig. 1). In our study of 23 pregnant small-pony mares, both eye width and eye length were measured. Of these two measurements, length was the better single predictor of days before parturition (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. Therefore, simply measuring eye length is sufficient.

After the length is recorded, we recommend that the image be unfrozen, and the scan and eye measurement repeated twice to obtain a total of three sets of length measurements. The average of the three values is the best predictor of DBP. However,

if the mare's behavior or other conditions make three separate measurements difficult, a single measurement of the length of the eye can be used to estimate DBP with minimal loss of accuracy.[4]

Transrectal examination of the mare's reproductive tract can efficiently be performed using portable ultrasound units equipped with a 5.0–7.5 mHz linear array transducers.[a] For field examinations, ultrasound units can be positioned within opaque cases to decrease sun glare.

3. Results

Previously reported data as well as more recent data from our laboratory indicate that transrectal measurement of fetal eye length can be used to estimate gestation length or DBP in equine pregnancies. Given the significant differences in birth weights and sizes of horse and pony foals, it is apparent that their growth profiles diverge at some point during gestation. The weights of horse and pony fetuses begin to diverge at ~100 days of gestation.[5] Therefore, beyond day 100, it can be presumed that fetal-eye measurements for light-horse fetuses will be proportionately larger than measurements from age-matched, small-pony fetuses. At this point, measurements of the horse fetal eyes are unlikely to accurately predict gestational ages of small-pony fetuses and vice versa. Normal ranges for fetal-eye measurements at different stages of gestation have been reported for light-horse breeds.[2] More recently, we have generated reference ranges for transrectal ultrasonographic measurements of fetal eyes to predict DBP in small ponies.[4] Additionally, we report here data that refines the previously reported light-horse ranges.

Pregnancies in Pony Mares

In our study, we evaluated 36 pregnancies over 2 yr in a group of 23 pony mares. These Shetland-type (100–250 kg) mares are maintained together with stallions in a semi-feral pony herd under natural social and breeding conditions and with minimal human contact. No routine breeding interventions or examinations are performed in this herd. During the course of our study, with few exceptions, breeding dates and dates of last ovulation were not systematically known. Monthly transrectal ultrasonographic examinations were conducted on pregnant study mares from December 2003 until parturition in April through July 2004 and from August 2004 until parturition in March through July 2005. All foals seemed to be full term at birth and were healthy with the exception of one foal from a primiparous mare that was extracted dead after a dystocia. No adverse effects of repeated ultrasound examinations were evident.

Mean eye-length measurements were obtained monthly as described above. After parturition, we calculated the DBP 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 measurements within pregnancy, repeated measurements across mares, and unbalanced study design. The nlme package in the R statistical language was used.[b6]

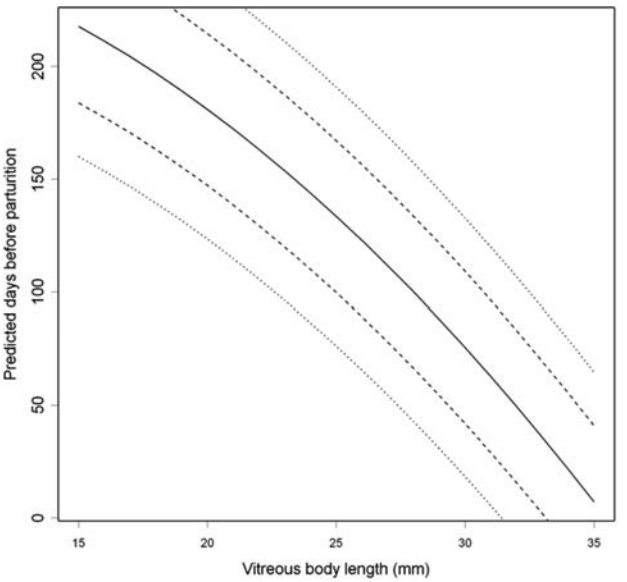

Fig. 2. Graphic depiction of regression curve (solid line) for eye length of small-pony fetuses showing 75% (dashed lines) and 95% (dotted lines) prediction intervals for DBP. Reprinted from *Theriogenology*, Vol 66, Turner, R.M., McDonnell, S.M., Feit, E.M., Grogan, E.H. and Foglia, R.F., Real-time ultrasound measure of the fetal eye (vitreous body) for prediction of parturition date in small ponies, 331–337, Copyright (2006), with permission from Elsevier.

The following regression equation was generated for pony-mare pregnancies:

$$\text{DBP} = 265.16 - 0.21 \times \text{vitreous body length in mm}^2$$

Figure 2 is a graphic depiction of the regression curve for pony-fetus eye lengths showing the 75% and 95% prediction intervals. Table 1 lists prediction intervals for DBP for eye lengths from 10 to 35 mm based on this model. Either the above equation, the graph in Figure 2, or the information in Table 1 can be used to readily estimate DBP after the pony fetal-eye length is determined.

A premise of our study was that small-pony fetuses would have smaller eye measurements and possibly different growth-curve characteristics than those previously published for light-horse mares. However, the design of our study required that we use DBP as our dependent variable (we did not know ovulation or breeding dates in most cases), whereas previously published data for light-horse mares used days of gestation (based on last-known breeding date) as the dependant variable. Therefore, we could not directly compare our results with the data

Table 1. Pony

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

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

for light-horse mares. However, in our study, there were four pregnancies for which we could establish gestational age based on ultrasonic appearance of the conceptus before 60 days gestation as described above. We compared eye measurements taken from these four mares (7 or 8 measurements at monthly examinations for each mare for a total of 29 measurements) with the regression line reported by Kahn and Leidl[2] for horse mares. Twenty-six of twenty-nine pony eye lengths fall below the regression line, indicating that, as suspected, pony fetal-eye measurements are smaller than those of light-horse mares. Because measurements of dispersion were not reported in the Kahn and Leidl[2] regression model, we can not statistically compare growth curves; however, 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 it plateaued at ~9 mo of gestation. This indicates that data for horse eye lengths can not be applied to pony eye lengths and vice versa.

Other Measures in Ponies

In our study of pony mares, year of parturition, foal gender, and size of mare had no significant impact on eye growth and size at parturition.

Parity of the mare (primiparous 2-yr-old fillies versus multiparous 3-yr-old or older fillies and mares) had a significant effect on the birth weight of the foal. Foals born from primiparous fillies were significantly smaller than those born from multiparous mares. However, eye length of fetuses of primiparous and multiparous mares within the last month before parturition were similar (independent t-test, 34 df, $p > 0.10$). For 18 foals for which birth weights were determined, the association of birth weight and eye length ($r = -0.10$, $p > 0.10$) within 1 mo of parturition was not significant.

For foals for which birth weights were available, foal weights for mares with body-condition scores <6 (n = 3) were significantly lower than those for mares with scores >6 (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 body-condition scores 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 body-condition score (Pearson $r = 0.11$).

Ease of Obtaining Fetal Eye Measures

We performed 273 examinations of 23 pregnant small-pony mares over a 2-yr period. An eye was successfully identified and measured in 91% of the examinations that were conducted in the third month of gestation or later. Failure to locate the eye was most frequent during months three (35% of examinations) and four (19% of examinations) of gestation, least frequent during months eight (2.5%), nine (0%), ten (8%), and eleven (0%), and intermediate during months five (12%), six (12%), and seven (13%). In early pregnancies, failure was typically attributed to low position of the fetal head and/or posterior presentation of the fetus at the time of the examination. During later gestation, failure was typically attributed to low position of the fetal head at the time of examination.[4] However, our data indicate that even under field conditions using portable ultrasound equipment, this procedure is very straightforward and has a high likelihood of producing the information necessary to estimate DBP.

Pregnancies in Horse Mares

Kahn and Leidl[2] previously reported a regression equation that can be used to predict gestation length based on fetal-eye measurements from horse-mare pregnancies.

This equation describes a linear model throughout gestation. We noticed that this light-horse mare model contained few data points beyond day 280 of gestation. Additionally, it differed from our pony-mare model in that our model was curvilinear, clearly plateauing during late gestation. Taking these observations together, we felt that additional measurements of light-horse mare fetal eyes during late gestation were required to determine if the light-horse mare regression equation was truly linear throughout gestation or if in fact it plateaued in a manner similar to the pony-mare curve.

Table 2. Light Horse

Eye Length (Vitreous Body Medial to Lateral, mm)	Day of Gestation		
	Mean	75% PI	95% PI
Kahn and Leidl, 1987 (n=77 TB and STB)			
10	86	65–107	50–123
11	92	72–112	57–127
12	98	78–117	64–131
13	104	85–123	71–136
14	110	91–129	78–142
15	116	98–134	85–148
16	122	104–141	91–154
17	129	111–147	98–160
18	135	117–153	104–166
19	142	124–160	111–173
20	149	131–166	118–179
21	155	137–173	124–186
22	162	144–180	131–193
23	169	151–187	138–200
24	176	158–194	145–207
25	183	165–201	152–214
26	190	172–208	159–221
27	198	179–216	166–229
28	205	187–223	173–237
29	212	194–231	180–245
30	220	201–239	187–253
McDonnell, Turner et al, in progress, 2006 (n=66 TB, STB, QH, Arabian, Warmblood)			
30	284	247–321	220–348
31	291	254–328	227–355
32	298	261–335	234–361
33	303	266–340	239–367
34	308	271–345	244–372
35	312	275–349	248–376
36	315	278–353	251–380
37	318	280–356	252–384

Prediction mean with 75% and 95% prediction interval ranges for day of gestation.

Fetal-eye measurements were obtained from the pregnancies of 66 light-horse mares (~400–550 kg) using the same methods as described above for pony mares. All horse mares had confirmed last breeding or ovulation dates; therefore, gestational age was definitively known and was ≥230 days. Fetal eye lengths were obtained at a single point during gestation for each mare. All foals were full term at birth and healthy.

Table 2 lists mean and prediction intervals for days of gestation for light-horse mares based on fetal eye length. The table represents the most current information available for comparing gestational age of horse-mare pregnancies to fetal-eye measurements. Kahn and Leidl's[2] data were used for eye lengths of 10–30 mm (early to mid gestation), and our recent data were used for lengths of 30–37 mm (mid to late gestation). Our data, which includes a larger data set for mid to late gestation, describes a curvilinear relationship between gestational age and eye length as we described for ponies. When taken together with our pony-mare data, it also indicates that, at any given point beyond ~100 days of gestation, horse fetal eyes are larger than pony fetal eyes.

4. Discussion

The goal of this abstract is to present a practical method that can be used by field practitioners to estimate gestation length in horse and pony mares. As part of this goal, we present newly developed reference values for transrectal ultrasonographic measurements of fetal eye lengths in small pony mares and refined reference ranges for horse mares. Beyond ~100 days of gestation, growth curves for horse and pony fetuses diverge, and therefore, different data sets are required for the two groups.

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. It causes no apparent damage to the fetus, even when performed multiple times over the course of a single pregnancy. Our pony study was done under field conditions with semi-feral mares. Even under these conditions, we experienced minimal difficulties when performing this procedure; we were able to locate the eye and obtain measurements in >90% of examination attempts beyond 2 mo of gestation.

Our regression analysis yielded a curvilinear model of growth for fetal eye length in pony fetuses. This was in contrast to a linear model reported for light-horse fetuses and fetuses of other species.[2,7–10] We noticed that the majority of the horse fetal measurements were taken in mid gestation, and relatively few were obtained beyond day 280 of gestation. Additionally, the authors reported that the fit to their linear model was fairly close in early and mid gestation but not in later gestation. To better define the relationship between gestation length and eye length in late gestation horse pregnancies, we obtained additional measurements of horse fetal eyes beyond day 280 of gestation. Our new data on late gestation horse mare pregnancies refine the previously reported model and show that the relationship between eye length and gestational age in horse mares is similar to the relationship in pony mares and is curvilinear rather than linear. A direct comparison of our data on horse-mare and pony-mare fetuses indicates that, at any given point during mid to late gestation, horse fetal eyes are larger than pony fetal eyes. Therefore, when examining horse and pony fetal orbits, the practitioner should compare his or her data with the appropriate curve.

Our study on pony-mare pregnancies differed from the Kahn and Leidl[2] study in which fetal eye measurements were obtained on a known day of gestation based on last breeding date. Because ovulation or last breeding dates were known with certainty in only four instances in our pony study, we used the date of parturition to retrospectively calculate the DBP on which each set of eye measurements had been obtained. The resulting prediction

intervals are wider compared with those reported for horse mares. Our prediction intervals reflect variation because of both the development of the foal and the natural wide variation in mare gestation length.[11,12] One might conclude that the wide intervals inherent to estimating DBP are less useful than the tighter intervals inherent to estimating day of gestation; however, the practical value of the two approaches are likely to be similar, because predicting DBP explicitly accounts for the natural variation in gestation length, whereas predicting day of gestation does not. Using our data for ponies (Table 1; Fig. 2) and either the 95% or 75% confidence intervals, the practitioner can choose to have a 95% chance of correctly estimating DBP over a relatively wide range of days or a 75% chance of correctly estimating DBP over a more narrow range of days.

Although foals from primiparous pony mares in our study weighed significantly less than foals from multiparous pony mares, eye measurements did not differ significantly between the two groups. Additionally, fetal eye measurements did not seem to be affected by the mare's body condition score (BCS) near parturition or the mare's height; however, 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 suggest that variations in fetal size within a breed might not be reflected in fetal eye measurements. Although we did not address breed differences in our study, the question arises as to whether our pony results would be useful for predicting DBP of fetuses from non–Shetland-type breeds of similar sizes and also whether the Kahn and Leidl[2] data (based largely on Thoroughbred and Standardbred mares) would be useful for predicting gestation length of fetuses from non-Thoroughbred and non–Standardbred-type horse 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 (e.g., Arabians compared with Quarter Horses), and therefore, it may confound application of prediction models among breeds, even among breeds of similar size.

Summary

1. Beyond ~100 days of gestation, the sizes of horse and pony fetuses differ. Therefore, to accurately predict gestation length or DBP beyond 100 days of gestation, different data sets are required for the two groups. Current data sets for both horse and pony fetal eyes are presented here.
2. In contrast to a previous report on horse fetal-eye measurements, we found that the growth curves for both horse and pony fetal eyes are curvilinear and not linear.
3. Transrectal ultrasonographic measurement of fetal eye length is a safe and straightforward field procedure that can be used to estimate gestation length or DBP from ~90 days of gestation until term.
4. When using fetal eye length to estimate DBP, the prediction interval is wider than when using fetal eye length to estimate days of gestation. This may be because estimating DBP inherently reflects variation caused by both development of the foal and the natural wide variation in mare gestation length. The practical values of both approaches are probably similar.
5. Using our data for ponies or horses, the practitioner can choose to have a 95% chance of correctly estimating DBP over a relatively wide range of days or a 75% chance of correctly estimating DBP over a more narrow range of days.

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[a]Sonovet 600 and Sonovet 2000 portable ultrasound machines, Universal Medical, Bedford, NY 10506.

[b]R version 2.010 with nlme version 3.1–65, 3.1–65 R Foundation, Vienna, Austria.