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Prediction of gestational age based on foetal ultrasonographic biometric measurements in light breed horses

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Abstract

A table was generated, based on foetal ultrasonographic measurements in light breed mares, for each day of gestation beginning with day 100, to provide the predicted value of four biometric parameters: biparietal diameter (BPD), eye approximated volume (EyV), foetal aortic diameter (AortD) and femur length (FL). Using this table, day of gestation was successfully predicted in 23 Quarter Horses (QH) with known mating or ovulation dates. BPD, EyV and FL were the best foetal age predictors between 100- and 200-days gestation predicting within 2 weeks of the actual day of gestation, while BPD and EyV were best between 200 and 300 days (within 3 weeks), and EyV was best after 300 days (within 3 weeks).

KEYWORDS
age, equine, foetus, QH, ultrasound

1 | INTRODUCTION

Assessment of foetal growth and age prediction by ultrasonography is routinely performed in human using established tables. In small animals, ultrasonographic foetal biometry of the head is commonly applied in dogs (Alonge et al., 2016; Kim et al., 2007; Lopate, 2008; Luvoni & Beccaglia, 2006) and cats (Beccaglia et al., 2008; Keiser et al., 2017) to allow prediction of parturition day and safe planning of elective caesarean section. In small ruminants (goat and sheep), cranium diameter measurements have been largely studied during most of the pregnancy in different breeds (Haibel, 1988; Haibel & Perkins, 1989; Haibel et al., 1989; Karen et al., 2009; Reichle & Haibel, 1991). In cattle, foetal biometric parameters are mostly evaluated during early to mid-gestation to estimate calving date (Adeyinka et al., 2014; Kähn, 1990; White et al., 1985; Wright et al., 1988). In mares, determination of the age of the pregnancy is well documented early in gestation based on the size of the embryonic vesicle, the yolk sac and allantoic sac ratio (Bergfelt & Adams, 2011; Ginther, 1998). Growth charts have been published showing the relationship between anatomical growth parameters and days of gestation in equine foetuses (Bucca et al., 2005; Hartwig et al., 2013; Hendriks et al., 2009; Kähn & Leidl, 1987; Murase et al., 2014; Renaudin et al., 2000; Turner et al., 2006a). To date, a few tables exist comparing days of gestation or days before parturition to eye length only, in ponies and light breed mares, but the prediction accuracy is not known (Hartwig et al., 2013; Turner et al., 2006b). Tables based on multiple biometric parameters would be very useful to the equine practitioner in the field in order to estimate foetal age when ovulation/breeding dates is unknown or to assess foetal growth when gestational age is known. The objectives of this study are: (1) to establish a table based on which foetal age can be predicted with multiple biometric parameters (phase 1), and (2) to assess the accuracy within weeks of age prediction in Quarter Horses (phase 2).

2 | MATERIAL AND METHODS

2.1 | Phase 1: Generating the gestational age prediction table

2.1.1 | Mares

Ten light breed healthy pregnant multiparous mares (Quarter Horses ($n = 7$) and Thoroughbred ($n = 3$)), aged 9–22 years, with known artificial insemination and ovulation dates were used. All mares were stabled at the Center for Equine Health (Davis, CA). Their diet included alfalfa and permanent pasture during the summer months. Grain ration supplements were given as required. The mares were vaccinated with Pneumabort K (equine herpes virus type 1) at 5, 7 and 9 months of gestation and were boosted with a four-way vaccine (influenza, tetanus and eastern and western encephalomyelitis) at day 315 of gestation.

2.1.2 | Ultrasonography

A single experienced operator scanned each mare by ultrasonography at 15-day intervals from 100 days gestation (day 0 = 1st day ovulation was detected by daily or every other day ultrasonography) to parturition. Mares were restrained in stocks and scanned fully awake. Ultrasonography was performed using a SonoAce 1500 scanning system equipped with real time 5.0 and 3.5 MHz linear transducers (Medison America). Transrectal ultrasonographic examinations were performed with a 5.0 MHz transducer. Transabdominal ultrasonography was initiated with a 5.0 MHz transducer followed by a 3.5 MHz transducer if greater depth penetration was required. An ultrasonographic coupling gel was applied to the abdomen of the mares after clipping the hair from the mammary glands to the xyphoid to obtain the best image quality.

During each examination, four foetal anatomical regions were observed and measured using a standardized scan plan as already described (Renaudin et al., 2000).

Cranium

It was imaged in cross section with the falx cerebri separating the 2 hemispheres symmetrically, when internal structures could be seen (Figure 1). The image was frozen when the maximal cross-sectional area of the cranium appeared oval. The cranium diameter also called biparietal diameter (BPD) was measured using calliper cursors positioned on the outer margin of the skull nearest the transducer and the inner margin of the skull further away from the transducer (Figure 1).

Eye

The eye was viewed in cross section. The anterior and posterior portions of the capsule of the lens served as anatomical landmarks (Figure 2). The image was frozen when the maximal cross-sectional diameter of the vitreous body was obtained. The eye depth (EyD) was measured by placing calliper cursors from the margin of the anterior portion of the capsule of the lens to the inner margin of the optic disc. The eye length (EyL) perpendicular to the eye depth was measured from the maximum length of the inner margins of the vitreous body. The approximate eye volume (EyV) was then calculated: EyV = EyL x EyL x EyD.

Aorta

It was observed in longitudinal section (Figure 3). The image was frozen when the most anterior portion of the aorta was seen in systole

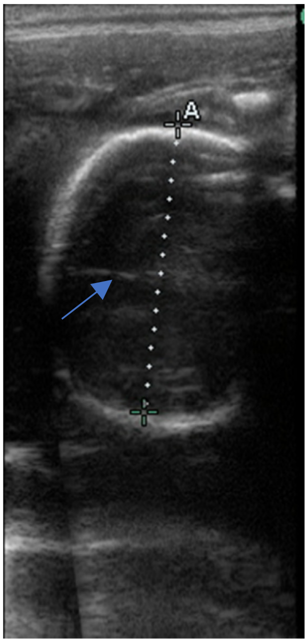

FIGURE 1 Sonogram of the cranium (in cross-sectional view) of a 185-days old Quarter Horses (QH) foetus: the falx cerebri separates the 2 hemispheres symmetrically (arrow). The BPD is measured using calliper cursors positioned from the outer margin of the skull nearest to the transducer to the inner margin of the skull furthest away from the transducer (distance between the two + = 51.9 mm). The ultrasound image was obtained using a 5 MHz linear probe via the transrectal approach

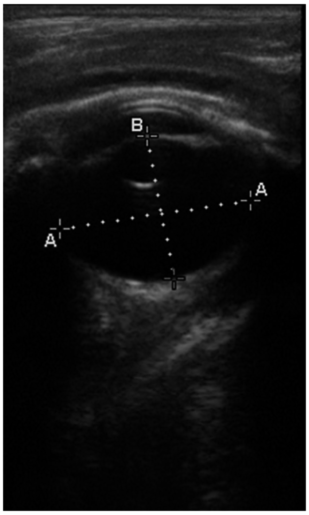

FIGURE 2 Sonogram of the eye (in cross-sectional view) of a 338-days old mule foetus: the anterior and posterior portion of the lens are visualized. The image is frozen when the maximal cross-sectional diameter of the vitreous body is obtained. Eye length (EyL) (maximum length of the inner margins of the vitreous body between A) and eye depth (EyD) (margin of the anterior portion of the capsule of the lens to the inner portion of the optic disc between B) perpendicular to EyL, are measured. Eye approximated volume (EyV) is calculated: EyL (33.6 mm) x EyL (33.6 mm) x EyD (25.3 mm) = 28,563 mm^2. The ultrasound image was obtained using a 5 MHz linear probe via the transrectal approach

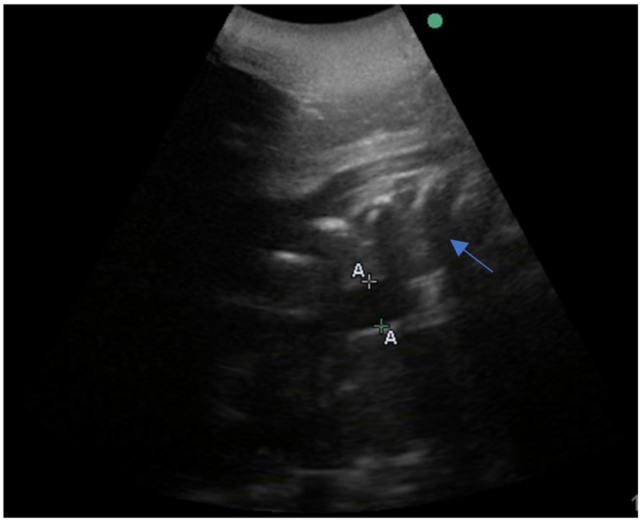

FIGURE 3 Sonogram of the aorta (in long view) of a 262-days old Quarter Horses (QH) foetus. The image is frozen when the most anterior portion of the aorta is seen in systole (maximum dilation of the aorta). The aortic diameter (AortD) is measured by placing the 2 calliper cursors on the vessel margins (between A = 18.5 mm). The round hyperechogenic ribs are seen with their characteristic acoustic shadows (arrow). Foetal head is to the left and tail to the right. The ultrasound image was obtained using a 3.5 MHz curvlinear probe via the transabdominal approach

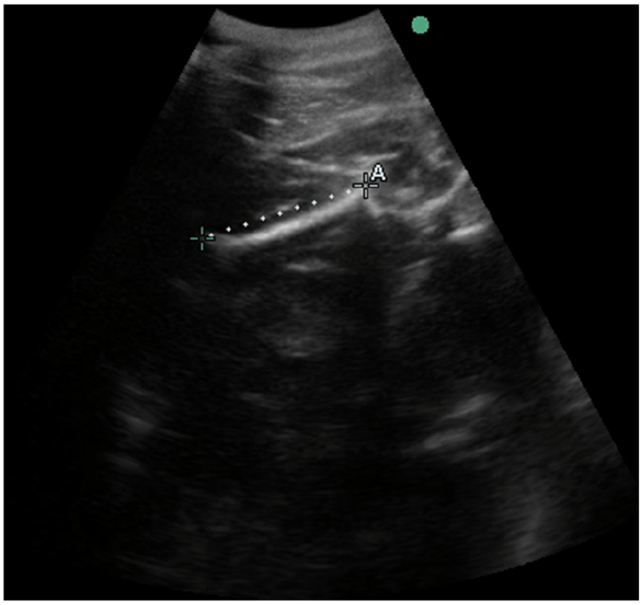

FIGURE 4 Sonogram of the femur (in long view) of a 185-days old Quarter Horses (QH) foetus. The image is frozen when the longest view of the ossified portion of the femur is obtained. Femur length (FL) is then measured with the calliper cursors (between A = 64 mm). The ultrasound image was obtained using a 3.5 MHz curvlinear probe via the transabdominal approach

(maximum dilation of the aorta). The aortic diameter (AortD) was measured by placing the two calliper cursors on the vessel margins of the most anterior portion of the aorta.

Femur

It was imaged in longitudinal view (Figure 4). The image was frozen when the longest view of the ossified portion of the femur was obtained. Femur length (FL) was measured using calliper cursors.

2.1.3 | Determination of foal growth and well-being

The birth of each foal was observed and the parameters time to suck, stand and nurse were recorded. A general physical examination including determination of body weight, temperature, pulse and respiratory rates and an IgG concentration test (IDEXX laboratory foal IgG test kit; IDEXX Laboratories Inc.) were performed on each foal on the day of birth. The health status of each foal was determined one week after parturition and each foal was weighted again at day 8 after parturition to assess growth rate (normal growth rate is 1.0–1.5 kg/day). Each placenta was grossly examined post foaling to determine whether there were any abnormalities.

2.1.4 | Statistical analysis

Least squares linear regression was used to model the relationship between the linear, and where appropriate (based on improvement of model fit) quadratic transformation of days of gestation to predict the dependent variables AortD, EyV, BPD and FL. Using the linear models, a table and growth charts were then generated for each day of gestation, beginning with day 100, that provides the least squares predicted mean of each of the four dependent variables (Table 1, Figure 5).

2.2 | Phase 2: Testing the generated prediction table

2.2.1 | Mares

Twenty-three healthy Quarter Horses (QH) pregnant mares aged 3–20 years, that were either research or client horses with known mating and ovulation dates were used.

2.2.2 | Ultrasonography

The same experienced ultrasonographer scanned all mares using a Sonosite Edge (Fujifilm Sonosite) equipped with a 5 MHz linear and a 3.5 MHz convex probes. Some mares who were part of a research project were ultrasounded regularly once a month, transrectally only, starting at 7 months (181–210 days) of gestation. Most client mares were scanned once using the transrectal and the transabdominal approaches as part of a foetal well-being examination. Depending on the presentation of the foetus and the approach used, some or all

TABLE 1 Predicted measurements of different biometric parameters (AortD: aortic diameter in mm; BPD, biparietal diameter in mm; EyV, eye approximated volume in mm^3; FL, femur length in mm) for each day of gestation (day) from 100- to 342 days of gestation based on bimonthly measurements of 10 light breed mares

Day	AortD	EyV	BPD	FL	Day	AortD	EyV	BPD	FL	Day	AortD	EyV	BPD	FL
100	3.00	1,522.60	26.81	14.29	127	5.63	4,617.00	37.29	29.88	154	8.26	7,711.41	46.95	45.47
101	3.10	1,637.21	27.21	14.87	128	5.73	4,731.61	37.66	30.46	155	8.36	7,826.02	47.29	46.04
102	3.20	1,751.81	27.61	15.45	129	5.83	4,846.22	38.03	31.04	156	8.46	7,940.62	47.63	46.62
103	3.29	1,866.42	28.01	16.03	130	5.93	4,960.83	38.40	31.61	157	8.56	8,055.23	47.97	47.20
104	3.39	1,981.03	28.41	16.60	131	6.02	5,075.43	38.77	32.19	158	8.65	8,169.84	48.31	47.78
105	3.49	2,095.64	28.81	17.18	132	6.12	5,190.04	39.14	32.77	159	8.75	8,284.45	48.65	48.35
106	3.59	2,210.24	29.21	17.76	133	6.22	5,304.65	39.50	33.34	160	8.85	8,399.05	48.99	48.93
107	3.68	2,324.85	29.60	18.34	134	6.31	5,419.26	39.87	33.92	161	8.95	8,513.66	49.32	49.51
108	3.78	2,439.46	30.00	18.91	135	6.41	5,533.86	40.23	34.50	162	9.04	8,628.27	49.66	50.09
109	3.88	2,554.07	30.39	19.49	136	6.51	5,648.47	40.60	35.08	163	9.14	8,742.88	49.99	50.66
110	3.98	2,668.67	30.78	20.07	137	6.61	5,763.08	40.96	35.65	164	9.24	8,857.49	50.32	51.24
111	4.07	2,783.28	31.17	20.64	138	6.70	5,877.69	41.32	36.23	165	9.34	8,972.09	50.65	51.82
112	4.17	2,897.89	31.56	21.22	139	6.80	5,992.30	41.68	36.81	166	9.43	9,086.70	50.98	52.39
113	4.27	3,012.50	31.95	21.80	140	6.90	6,106.90	42.04	37.39	167	9.53	9,201.31	51.31	52.97
114	4.37	3,127.11	32.34	22.38	141	7.00	6,221.51	42.40	37.96	168	9.63	9,315.92	51.64	53.55
115	4.46	3,241.71	32.73	22.95	142	7.09	6,336.12	42.75	38.54	169	9.72	9,430.52	51.97	54.13
116	4.56	3,356.32	33.11	23.53	143	7.19	6,450.73	43.11	39.12	170	9.82	9,545.13	52.29	54.70
117	4.66	3,470.93	33.50	24.11	144	7.29	6,565.33	43.46	39.69	171	9.92	9,659.74	52.62	55.28
118	4.76	3,585.54	33.88	24.69	145	7.39	6,679.94	43.82	40.27	172	10.02	9,774.35	52.94	55.86
119	4.85	3,700.14	34.27	25.26	146	7.48	6,794.55	44.17	40.85	173	10.11	9,888.95	53.26	56.44
120	4.95	3,814.75	34.65	25.84	147	7.58	6,909.16	44.52	41.43	174	10.21	10,003.56	53.58	57.01
121	5.05	3,929.36	35.03	26.42	148	7.68	7,023.76	44.87	42.00	175	10.31	10,118.17	53.90	57.59
122	5.15	4,043.97	35.41	26.99	149	7.78	7,138.37	45.22	42.58	176	10.41	10,232.78	54.22	58.17
123	5.24	4,158.57	35.78	27.57	150	7.87	7,252.98	45.57	43.16	177	10.50	10,347.38	54.54	58.75
124	5.34	4,273.18	36.16	28.15	151	7.97	7,367.59	45.92	43.74	178	10.60	10,461.99	54.85	59.32
125	5.44	4,387.79	36.54	28.73	152	8.07	7,482.19	46.26	44.31	179	10.70	10,576.60	55.17	59.90
126	5.54	4,502.40	36.91	29.30	153	8.17	7,596.80	46.61	44.89	180	10.80	10,691.21	55.48	60.48
Day	**AortD**	**EyV**	**BPD**	**FL**	**Day**	**AortD**	**EyV**	**BPD**	**FL**	**Day**	**AortD**	**EyV**	**BPD**	**FL**
181	10.89	10,805.81	55.80	61.05	208	13.52	13,900.22	63.83	76.64	235	16.15	16,994.62	71.05	92.23
182	10.99	10,920.42	56.11	61.63	209	13.62	14,014.83	64.11	77.22	236	16.25	17,109.23	71.30	92.80
183	11.09	11,035.03	56.42	62.21	210	13.72	14,129.43	64.39	77.80	237	16.35	17,223.84	71.55	93.38

TABLE 1 (Continued)

Day	AortD	EyV	BPD	FL	Day	AortD	EyV	BPD	FL	Day	AortD	EyV	BPD	FL
184	11.19	11,149.64	56.73	62.79	211	13.82	14,244.04	64.67	78.37	238	16.45	17,338.45	71.80	93.96
185	11.28	11,264.24	57.04	63.36	212	13.91	14,358.65	64.95	78.95	239	16.54	17,453.06	72.05	94.54
186	11.38	11,378.85	57.35	63.94	213	14.01	14,473.26	65.23	79.53	240	16.64	17,567.66	72.30	95.11
187	11.48	11,493.46	57.65	64.52	214	14.11	14,587.87	65.51	80.10	241	16.74	17,682.27	72.55	95.69
188	11.58	11,608.07	57.96	65.10	215	14.21	14,702.47	65.78	80.68	242	16.84	17,796.88	72.79	96.27
189	11.67	11,722.68	58.26	65.67	216	14.30	14,817.08	66.06	81.26	243	16.93	17,911.49	73.03	96.85
190	11.77	11,837.28	58.57	66.25	217	14.40	14,931.69	66.33	81.84	244	17.03	18,026.09	73.28	97.42
191	11.87	11,951.89	58.87	66.83	218	14.50	15,046.30	66.60	82.41	245	17.13	18,140.70	73.52	98.00
192	11.97	12,066.50	59.17	67.40	219	14.60	15,160.90	66.87	82.99	246	17.23	18,255.31	73.76	98.58
193	12.06	12,181.11	59.47	67.98	220	14.69	15,275.51	67.14	83.57	247	17.32	18,369.92	74.00	99.15
194	12.16	12,295.71	59.77	68.56	221	14.79	15,390.12	67.41	84.15	248	17.42	18,484.52	74.24	99.73
195	12.26	12,410.32	60.07	69.14	222	14.89	15,504.73	67.68	84.72	249	17.52	18,599.13	74.47	100.31
196	12.36	12,524.93	60.36	69.71	223	14.99	15,619.33	67.94	85.30	250	17.62	18,713.74	74.71	100.89
197	12.45	12,639.54	60.66	70.29	224	15.08	15,733.94	68.21	85.88	251	17.71	18,828.35	74.95	101.46
198	12.55	12,754.14	60.95	70.87	225	15.18	15,848.55	68.47	86.45	252	17.81	18,942.95	75.18	102.04
199	12.65	12,868.75	61.24	71.45	226	15.28	15,963.16	68.74	87.03	253	17.91	19,057.56	75.41	102.62
200	12.74	12,983.36	61.54	72.02	227	15.38	16,077.76	69.00	87.61	254	18.01	19,172.17	75.64	103.20
201	12.84	13,097.97	61.83	72.60	228	15.47	16,192.37	69.26	88.19	255	18.10	19,286.78	75.87	103.77
202	12.94	13,212.57	62.12	73.18	229	15.57	16,306.98	69.52	88.76	256	18.20	19,401.38	76.10	104.35
203	13.04	13,327.18	62.41	73.75	230	15.67	16,421.59	69.78	89.34	257	18.30	19,515.99	76.33	104.93
204	13.13	13,441.79	62.69	74.33	231	15.77	16,536.19	70.03	89.92	258	18.40	19,630.60	76.56	105.50
205	13.23	13,556.40	62.98	74.91	232	15.86	16,650.80	70.29	90.50	259	18.49	19,745.21	76.78	106.08
206	13.33	13,671.00	63.26	75.49	233	15.96	16,765.41	70.54	91.07	260	18.59	19,859.81	77.01	106.66
207	13.43	13,785.61	63.55	76.06	234	16.06	16,880.02	70.80	91.65	261	18.69	19,974.42	77.23	107.24
Day	**AortD**	**EyV**	**BPD**	**FL**	**Day**	**AortD**	**EyV**	**BPD**	**FL**	**Day**	**AortD**	**EyV**	**BPD**	**FL**
262	18.79	20,089.03	77.46	107.81	289	21.42	23,183.44	83.05	123.40	316	24.05	26,277.84	87.82	138.99
263	18.88	20,203.64	77.68	108.39	290	21.51	23,298.04	83.24	123.98	317	24.14	26,392.45	87.98	139.56
264	18.98	20,318.25	77.90	108.97	291	21.61	23,412.65	83.43	124.56	318	24.24	26,507.06	88.14	140.14
265	19.08	20,432.85	78.12	109.55	292	21.71	23,527.26	83.62	125.13	319	24.34	26,621.66	88.30	140.72
266	19.18	20,547.46	78.34	110.12	293	21.81	23,641.87	83.80	125.71	320	24.44	26,736.27	88.46	141.30
267	19.27	20,662.07	78.55	110.70	294	21.90	23,756.47	83.99	126.29	321	24.53	26,850.88	88.62	141.87
268	19.37	20,776.68	78.77	111.28	295	22.00	23,871.08	84.18	126.86	322	24.63	26,965.49	88.77	142.45

TABLE 1 (Continued)

Day	AortD	EyV	BPD	FL	Day	AortD	EyV	BPD	FL	Day	AortD	EyV	BPD	FL
269	19.47	20,891.28	78.98	111.85	296	22.10	23,985.69	84.36	127.44	323	24.73	27,080.09	88.93	143.03
270	19.56	21,005.89	79.20	112.43	297	22.20	24,100.30	84.55	128.02	324	24.83	27,194.70	89.08	143.61
271	19.66	21,120.50	79.41	113.01	298	22.29	24,214.90	84.73	128.60	325	24.92	27,309.31	89.23	144.18
272	19.76	21,235.11	79.62	113.59	299	22.39	24,329.51	84.91	129.17	326	25.02	27,423.92	89.38	144.76
273	19.86	21,349.71	79.83	114.16	300	22.49	24,444.12	85.09	129.75	327	25.12	27,538.52	89.53	145.34
274	19.95	21,464.32	80.04	114.74	301	22.58	24,558.73	85.27	130.33	328	25.22	27,653.13	89.68	145.91
275	20.05	21,578.93	80.25	115.32	302	22.68	24,673.33	85.45	130.91	329	25.31	27,767.74	89.83	146.49
276	20.15	21,693.54	80.46	115.90	303	22.78	24,787.94	85.62	131.48	330	25.41	27,882.35	89.98	147.07
277	20.25	21,808.14	80.66	116.47	304	22.88	24,902.55	85.80	132.06	331	25.51	27,996.95	90.12	147.65
278	20.34	21,922.75	80.87	117.05	305	22.97	25,017.16	85.97	132.64	332	25.61	28,111.56	90.27	148.22
279	20.44	22,037.36	81.07	117.63	306	23.07	25,131.76	86.15	133.21	333	25.70	28,226.17	90.41	148.80
280	20.54	22,151.97	81.27	118.20	307	23.17	25,246.37	86.32	133.79	334	25.80	28,340.78	90.55	149.38
281	20.64	22,266.57	81.47	118.78	308	23.27	25,360.98	86.49	134.37	335	25.90	28,455.38	90.69	149.96
282	20.73	22,381.18	81.68	119.36	309	23.36	25,475.59	86.66	134.95	336	25.99	28,569.99	90.83	150.53
283	20.83	22,495.79	81.87	119.94	310	23.46	25,590.19	86.83	135.52	337	26.09	28,684.60	90.97	151.11
284	20.93	22,610.40	82.07	120.51	311	23.56	25,704.80	87.00	136.10	338	26.19	28,799.21	91.11	151.69
285	21.03	22,725.00	82.27	121.09	312	23.66	25,819.41	87.17	136.68	339	26.29	28,913.82	91.25	152.26
286	21.12	22,839.61	82.47	121.67	313	23.75	25,934.02	87.33	137.26	340	26.38	29,028.42	91.38	152.84
287	21.22	22,954.22	82.66	122.25	314	23.85	26,048.63	87.50	137.83	341	26.48	29,143.03	91.52	153.42
288	21.32	23,068.83	82.85	122.82	315	23.95	26,163.23	87.66	138.41	342	26.58	29,257.64	91.65	154.00

the foetal biometric parameters (described above in phase 1) were measured and recorded.

This project was performed adhering to the Principles of Veterinary Medical Ethics of the AVMA (https://www.avma.org/KB/Policies/Pages/Principles-of-Veterinary-Medical-Ethics-of-the-AVMA.aspx) and under the AICUC protocol number 21694 for trans-abdominal foetal assessment.

2.2.3 | Statistical analysis

At each examination, for each biometric measurement, except for EyL, a predicted day of gestation was extracted from Table 1, established from measurements with horses in phase 1. For EyL measurements, the predicted day of gestation was calculated from the equation established by Hartwig et al. (2013): $y = 8.3756x + 11.90$, where y represents the gestational age in days and x represents the diameter of the foetal ocular orbit in millimetres. The actual days of gestation minus the model-predicted days of gestation (delta) in each individual were compared for each technique separately for 50-day increments of gestation (e.g. 101–150 days, 151–200 days, etc.) to determine if certain techniques were more effective in predicting the actual day of gestation than others at different stages of pregnancy. For example, if the actual day of gestation was 186 days, and the table generated day of gestation was 190 days, then the difference (delta) was – 4 days. Because some horses had more than one measurement taken in one or more 50-day periods, random effects analysis of variance was used to compare the delta values of each technique, with horse treated as the random effect. Post hoc comparisons were performed using Bonferroni multiple comparison adjustments.

The most effective technique(s) in predicting the day of gestation was (were) defined by being that (those) with the smallest mean delta(s). A difference was considered statistically significant when $p < .05$.

3 | RESULTS

3.1 | Phase 1. Gestational age prediction table

The ten mares delivered ten normal healthy foals without any assistance and their placenta looked grossly normal. IgG concentrations were >800 mg/dl 12 hr after parturition for all the foals. Foal birth weight ranged from 40 to 64 kg and all foals gained a normal amount of weight (10–16 kg) during the first week after parturition.

Based on the biometric measurements obtained for each mare every 2 weeks starting approximately 100 days of gestation, the predicted measurements of AortD, EyV, BPD and FL were calculated for each day of gestation (from 100 to 350 days gestation) and reported in Table 1.

3.2 | Phase 2. The generated table accurately predicted the gestational ages

3.2.1 | 101–150 days gestation

Based on 57 measurements, there were no significant differences between BPD, EyV, AortD and FL in predicting the day of gestation. However, EyL was significantly different ($p < .001$) from the other biometric measurements. FL, EyV, AortD, BPD and EyL could predict the day of gestation within 7, 8, 10, 11 and 33 days of the actual gestation date respectively. Predicted means and 95% confidence intervals in days are shown in Table 2.

3.2.2 | 151–200 days gestation

Based on 53 measurements, there were no significant differences between BPD, EyV, AortD and FL in predicting the day of gestation. EyL was significantly different ($p < .001$) from all the other biometric measurements. FL, EyV, BPD, AortD and EyL could predict the day of gestation within 10, 11, 14, 20 and 24 days of the actual gestation date respectively. Predicted means and 95% confidence intervals in days are shown in Table 2.

3.2.3 | 201–250 days gestation

Based on 38 measurements, there was no significant difference between BPD and EyV in predicting the day of gestation. FL was significantly different ($p < .05$) from all the other biometric measurements. BPD, EyV, EyL, AortD and FL could predict the day of gestation within 16, 19, 21, 27 and 31 days of the actual gestation date respectively. Predicted means and 95% confidence intervals in days are shown in Table 2.

3.2.4 | 251–300 days gestation

Based on 50 measurements, there was no significant difference between all biometric measurements in predicting the day of gestation. EyV, EyL, BPD, AortD and FL could predict the day of gestation within 13, 16, 21, 25 and 44 days of the actual gestation date respectively. Predicted means and 95% confidence intervals in days are shown in Table 2.

3.2.5 | 301–355 days gestation

Femur length variable could not be assessed due to the size of the femur, which exceeded the length of the ultrasound image at this stage of gestation. Based on 21 measurements, there were no significant difference between BPD, EyV, EyL and AortD in predicting

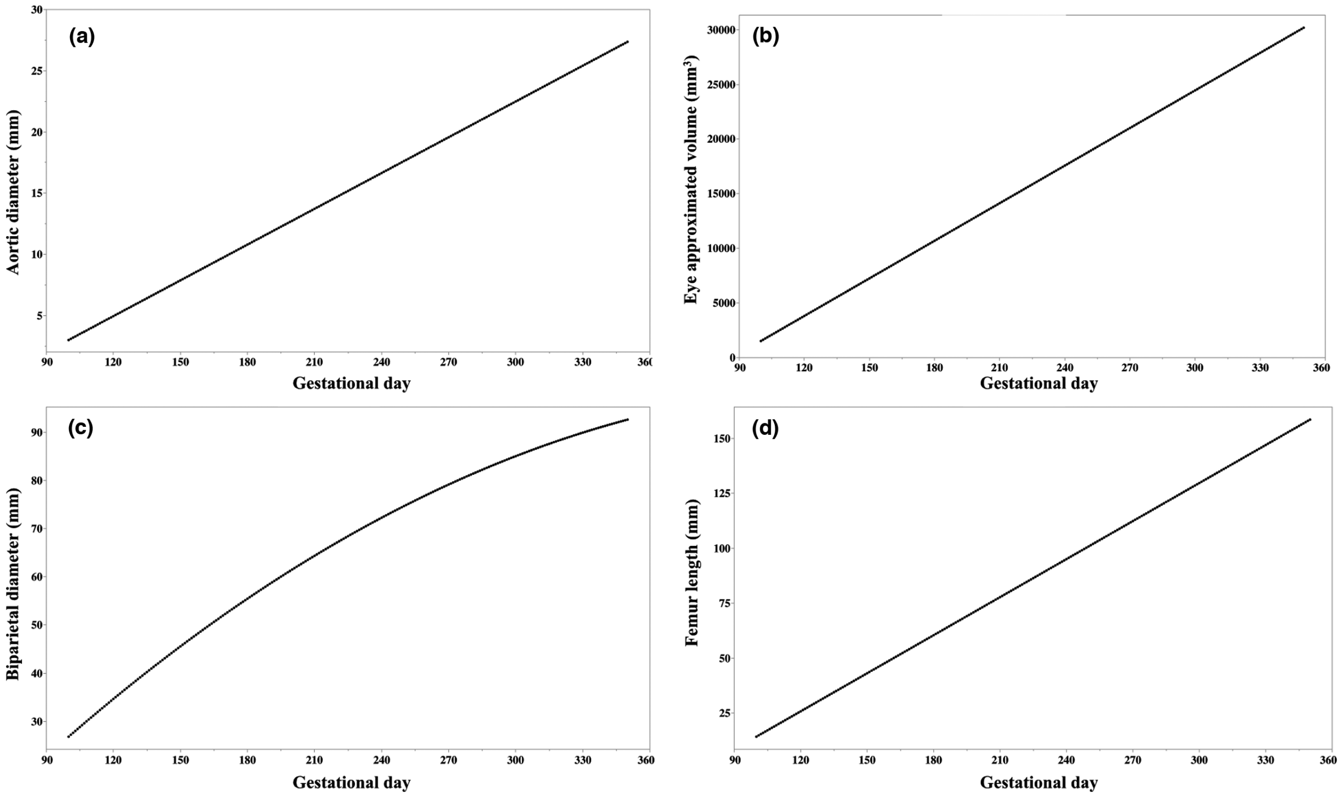

FIGURE 5 Growth charts of predicted measurement of aortic systolic diameter in mm (a), eye approximated volume in mm^3 (b), biparietal diameter in mm (c) and femur length in mm (d) from 100- to 350-days gestation based on bimonthly measurements of 10 light breed mares

TABLE 2 Predicted mean = *M* and 95% CI in days for foetal FL, femur length; EyV, eye approximated volume; EyL, eye length; BPD, biparietal diameter and AortD, aortic diameter for prediction of gestational day from 101 to 350 days of gestation in a group of 23 QH mares

Days of gestation	FL *M* (95% CI) in days	EyV *M* (95% CI) in days	EyL *M* (95% CI) in days	BPD *M* (95% CI) in days	AortD *M* (95% CI) in days
101–150	+2 (−3 to +7)	+3 (−3 to +8)	−27 (−33 to −22)	+6 (+1 to +11)	+3 (−3 to +10)
151–200	+5 (0 to +10)	+6 (+1 to +11)	−19 (−24 to −14)	+9 (+4 to +14)	+14 (+9 to +20)
201–250	−17 (−31 to −5)	+8 (−2 to +19)	−10 (−21 to 0)	+6 (−4 to +16)	+16 (+6 to +27)
251–300	−19 (−6 to +44)	−2 (−13 to +9)	+5 (−6 to +16)	+10 (−2 to +21)	+13 (+1 to +25)
301–350	NA	0 (−15 to +15)	+38 (+23 to +53)	+14 (−5 to +34)	+24 (+3 to +46)

the day of gestation. EyV, BPD, AortD and EyL could predict the day of gestation within 15, 34, 46 and 53 days of the actual gestation date respectively. Predicted means and 95% confidence intervals in days are shown in Table 2.

The accuracy of the different biometric parameters to predict gestational age within weeks for 50-day increments after 100 days of gestation is summarized in Table 3.

4 | DISCUSSION

Based on measurements performed every two weeks on a homogeneous group of light breed mares (phase 1), it was possible to generate a table (Table 1) of predicted values for four biometric variables (FL, BPD, EyV, AortD) from 100- to 342-days gestation. As all foals were healthy at birth, Table 1 allows assessment of normal foetuses and pregnancies. Two other tables exist in the horse literature that compare days of gestation or days before parturition to one variable (eye length) only, in two breeds that include Shetland ponies (Turner et al., 2006b) and Crioulo mares (Hartwig et al., 2013), but their accuracy is not known. Table 1 is based on one (Renaudin et al., 2000) of a few longitudinal studies (Hendriks et al., 2009; Murase et al., 2014) that used twice monthly measurements for a long period of time in gestation.

Landmarks are critical when measuring foetal parts with ultrasound. In this study the eye depth was measured from the anterior portion of the lens to the optic disc. This is different from landmarks used by Bucca et al. (2005), Bucca (2011), Murase et al. (2014) and Lanci et al. (2019) where eye depth is measured from the cornea to the optic disc. While referring to a table, proper landmarks should be used in order to draw conclusion on foetal growth and development.

TABLE 3 Prediction of gestational age (GA) within weeks based on the different biometric parameters used from 101 to 350 days of gestation in QH (FL, femur length; EyV, eye approximated volume; EyL, eye length; BPD, biparietal diameter; AortD, aortic diameter)

Prediction GA	101–150 days gestation	151–200 days gestation	201–250 days gestation	251–300 days gestation	301–350 days gestation
Within 1 week	**FL**				
Within 2 weeks	**EyV, AortD, BPD,**	**FL, EyV, BPD,**		**EyV**	
Within 3 weeks		AortD	**BPD, EyV, EyL**	**BPD, EyL**	**EyV**
Within 4 weeks		EyL	AortD	AortD	
>4 weeks	EyL		FL	FL	BPD, AortD, EyL

Note: Biometric parameters shown in bold have predicted means of no more than 10 days for a given 50-day period.

Using Table 1, gestational ages of 23 QH foetuses were predicted, within 2 weeks from 100 to 200 days gestation (with FL, EyV or BPD) and within 3 weeks from 201 to 300 days (with BPD, EyV) and after 301 days (with EyV; Table 3). FL, closely followed by EyV and BPD, was the most effective biometric variables in predicting foetal age from 100- to 200-days gestation with the smallest predicted mean (Table 2). It could predict foetal age within a week from 100- to 150-days, and within two weeks from 151- to 200-days of gestation (Table 3). After 200 days of gestation, FL was not very accurate, likely because the maximum length of the ossified portion of the femur becomes more and more difficult to measure due to its size, which eventually exceeded the length of the ultrasound image after 260 days of gestation (Renaudin et al., 2000). Similarly, BPD cannot generally be entirely imaged after 304 days of gestation and, therefore, cannot be used late in gestation as already reported (Renaudin et al., 2000). Most variables (BPD, AortD, EyV (prior to 250-days gestation) and FL (between 100- and 200-days gestation)) were underestimated while EyV was either over or underestimated after 250 days.

As opposed to bone measurements (FL and BPD), the aortic diameter can sometime be difficult to measure. In the author's experience, the acoustic shadow created by the foetal scapula prevents viewing the most anterior portion of the aorta. Therefore, in that instance, aortD is measured more caudally and is underestimated. However, the major advantage of this variable is that it is measurable consistently throughout the pregnancy. In this study, aortD could predict foetal age at best, within two weeks between 100- and 150 days of gestation, but as gestation advanced, this variable when available, was within three weeks during the next 50-days and more than three weeks after 200-days. The lack of efficiency of aortD in predicting foetal age, especially late in gestation, may be explained by the fact that this variable depends not only on foetal age but also on the foal's body measurements and on mare characteristics. Aortic diameter has been shown to be significantly correlated with neonatal foal weight, girth and hip height in normal pregnancies in QH and TB (Adams-Brendemuehl & Pipers, 1987; Reef et al., 1995) and with maternal prepartum weight in TB (Reef et al., 1995). In Dutch Warmbloods, it was found not to be influenced by parity but by maternal age; foetal aortD in mares 15 years of age or older was lower than in younger mares (Hendriks et al., 2009). In that study, however, the differences in foetal aortD were not reflected by significant differences in foal birth weight nor gestation length (Hendriks et al., 2009). Based on what is known, and on our study, aortD does not seem to be the most accurate foetal age predictor as opposed to FL, BPD and EyV.

Foetal age prediction based on EyL measurements was determined in our study using the formula established by Hartwig (Hartwig et al., 2013) in Crioulo mares. Using this model, EyL could never predict age within 2 weeks in QH at any time during gestation. EyL was as good predictor as BPD, but only from 200- to 300-days gestation predicting age within 3 weeks. Although Crioulo mares are smaller and weigh less than light horses (TB and Trotters), they have a foetal ocular orbit significantly larger than those of the light horses (Hartwig et al., 2013). In our study, EyL always overestimated foetal age (negative delta) before 250 days and mostly underestimated after 300 days (positive delta). If this formula is useful in the Crioulo breed, it may not be in other breeds like QH. Ponies foetal orbit have also been described to be smaller than light horses (Turner et al., 2006a, 2006b). Based on the Hartwig and Turner studies, foetal orbit is unlikely to be related to the size of the animal because there seems to be differences in developmental patterns across breeds (Hartwig et al., 2013). Foetal eye size near parturition does not vary directly with size of foal within this limited size range and suggests that variations in foetal size within a breed may not be reflected in foetal eye measurements (Turner et al., 2006b). In our study, EyV was a better variable than EyL up to 300 days in gestation in QH and could predict age within three weeks.

Prediction of foetal age has two purposes. The first one is to give an estimate of the age of the pregnancy or the parturition date when pregnant mares are presented for examinations without breeding or ovulation dates. According to this study, foetal age is best predicted during mid-gestation. Because gestation length is so variable in mares (310–374 days) (Howel & Rollins, 1951; Rossdale & Ricketts, 1974), it will always be an approximate estimate. Among the known factors influencing gestational length are foetal gender, daylight, mare's parity and age. It is accepted that male-product pregnancies are slightly longer (2–3 days of mean) than female counterparts (Satué et al., 2011). Mares have longer gestation when daylight period is shorter (Astudillo et al., 1960) and pregnant mares subjected to 16 hr of daily light from the beginning of December, advanced the date of parturition (Hodge et al., 1982). Age and multiparous state slow intra uterine growth (Satué et al., 2011), which may be attributable to a progressive degenerative change in the endometrium, and consequently, gestation length increases (Satué et al., 2011). Conversely, primiparous mares, which are usually young mares, have longer gestation because they are not anatomically and/or physiologically prepared (Satué et al., 2011). The size of the foal is influenced by mare's parity with primiparous mares producing smaller foals compared to multiparous ones (Robles, Dubois, et al., 2018; Robles, Peugnet, et al., 2018). In our study, information regarding mare's age and parity or foal sex and size were not considered as not all data was known for each individual horse.

The second purpose of foetal age prediction is to assess foetal growth when actual day of gestation is known. Foetal growth depends on adequate placental development and in normal pregnancy, foetal size and placental surface area are correlated (Bucca et al., 2005; Robles, Peugnet, et al., 2018). Therefore, if ultrasound biometric measurements determine that a foetus grows as predicted for gestational age, it is a sign that the placenta and the foetus are likely healthy. Conversely, if a foetus looks small for gestational age based on serial measurements of multiple biometric parameters, it may be a sign of intra uterine growth retardation (if no twin is present), and further investigations on the well-being of the foetus and placenta (equine biophysical profile; Adams-Brendemuehl & Pipers, 1987; Reef et al., 1995; Renaudin, 2018) or on the mare's health or body condition should be performed.

5 | CONCLUSION

We have established a table (Table 1) based on which foetal age can be predicted with multiple biometric parameters (FL, BPD, EyV, AortD). Accuracy of age prediction was assessed in QH, using the 4 variables in Table 1 as well as EyL based on Hartwig's model (Hartwig et al., 2013). The results of this study suggest that foetal age/growth in QH can be assessed within 1 week from 100- to 150-day gestation using FL, within 2 weeks from 100- to 200-days gestation using FL, BPD or EyV and within 3 weeks thereafter using EyV. As QH are light horses, table 1 may be useful with other light breed horses like Thoroughbred and Standardbred but more studies need to be done to assess prediction accuracy. Ideally, breed-specific tables should be generated.

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CONFLICT OF INTEREST

None of the authors have any conflict of interest to declare.

AUTHOR CONTRIBUTIONS

Catherine Renaudin designed the project, performed the research project and wrote the paper. Philip Kass analysed the data. Jean-Francois Bruyas helped in writing the paper.

DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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