# Calving date prediction based on transrectal ultrasonography determination of gestational age in beef cattle

R.K. Kasimanickam,<sup>a</sup> Rabie L. Abdel Aziz,<sup>a,c</sup> V.R. Kasimanickam,<sup>a,b</sup> C. Formen<sup>d</sup> <sup>a</sup>Department of Veterinary Clinical Sciences, <sup>b</sup>School of Molecular Biosciences, College of Veterinary Medicine, Washington State University, Pullman, WA; <sup>c</sup>Department of Theriogenology, Faculty of Veterinary Medicine, Beni-Suef University, Beni-Suef 62512, Egypt; <sup>d</sup>Trinity farm, Ellensburg, WA

## Abstract

In cattle, accurate gestation length determination during pregnancy diagnosis helps to determine calving date. This aids beef cattle producers to estimate length of calving season, labor availability and labor cost and to make nutritional management decisions. The objective of the study was to determine the error rates for projected calving date based on ultrasound determined gestational age in comparison to actual gestational age (derived as the number of days from artificial insemination [AI] date). Reproductive tract ultrasound examinations were performed in 8887 Angus cross beef females that were artificially inseminated in 12 spring calving herds between 2011 and 2016 to determine gestational age of the embryo or fetus. Only pregnancies estimated to be between 30 and 120 days (n = 6355 heifers and n = 2532 cows) were used in this study. The projected calving dates for actual and ultrasonography based gestational ages were determined using gestational length for beef cattle breed. Actual calving date, number of calves delivered at birth, gender of calf born, calf weight, and incidence of assisted birth were recorded. In heifers, the error rates for calving date prediction for actual and ultrasonography gestational ages were 22.1 and 9.9% respectively (P<0.0001); whereas in cows the error rates for actual and ultrasonography gestational ages were 13.5 and 9.1% respectively (P<0.01). The results of the multivariate analysis revealed dam's age and sire's calving ease estimated progeny difference (EPD) score influenced the accuracy of calving date prediction based on ultrasound determined gestational age (P<0.01). In conclusion, ultrasound determination of calving prediction was more accurate than traditional breeding date based calving prediction.

Keywords: Beef female, pregnancy diagnosis, ultrasonography, calving, error rate

## Introduction

In bovine practice, various techniques have been successfully adopted for the diagnosis of pregnancy. The most commonly used techniques are per-rectal palpation of the reproductive tract and transrectal ultrasonography of the reproductive tract and its contents.<sup>1-5</sup> These two methods are direct, reliable and fairly quick methods for diagnosis of pregnancy. Other methods in use are estimation of progesterone,<sup>5-7</sup>, pregnancy specific protein<sup>5.8</sup> and early conception factors<sup>9</sup> concentrations in serum.

Under most on-farm conditions, pregnancy can be rapidly and accurately diagnosed using ultrasound as early as 26 days after breeding.<sup>10</sup> Sensitivity and specificity of pregnancy diagnosis in lactating dairy cows based on ultrasonographic detection of uterine fluid as well as embryonic membranes from 28 to 35 days after AI was 96% and 97%, respectively.<sup>11</sup> Use of ultrasound to diagnose pregnancy offers several advantages over rectal palpation such as accurate earlier pregnancy diagnosis, establishing fetal age, determination of fetal gender, and identifying abnormalities in embryonic or fetal development. Sizes of maternal, placental and fetal components evaluated during ultrasonography examination have been used to explain reliable parameters to predict gestational age in cattle.<sup>9,12-14</sup> The manifestation of a heartbeat, crown rump length, occipitonasal length, orbital diameter, abdominal diameter, umbilical cord diameter, amniotic sac diameter, thoracic diameter, head circumference, and chest depth of the fetus and placentome size are all proposed approaches to estimate gestational age.<sup>9,12-18</sup>

Studies have shown that several factors contribute to the growth rate of developing embryo and fetus and that growth rate is not always uniform. Both maternal and embryonic or fetal factors, as well as farm management factors, can contribute to less than perfect prediction. In addition, *in utero* development may be linked to increased risk of smaller sized calves<sup>12,13</sup> or associated with larger birth size. Twin calves, calves born to primiparous cows and heifer calves tend to be lighter at birth compared

to singletons, calves born to multiparous cows, or bull calves respectively.<sup>15-17</sup> Poor maternal nutrition or reduced uterine capacity can limit conceptus growth.<sup>19.20</sup> In addition fetuess in larger offspring syndrome, an overgrowth disorder caused by assisted reproductive technologies in ruminants, featured with excessive birth weight.

Pregnancy is a measure of success for any breeding programs of beef cattle. Non-pregnant cows need to be identified as early as possible after the breeding season in order to determine reproductive efficiency, pregnancy rate and for culling decisions. Further, accurate gestation length determination helps to determine calving date which helps producer to estimate length of calving season, labor availability and labor cost. This also assists producers to make nutritional management preferences for the pregnant females.

The objective of the study was to determine the error rates for projected calving date for ultrasound determined gestational age in comparison to actual gestational age (derived as the number of days from the most recent AI date).

## Materials and methods:

#### Pregnancy diagnosis

Reproductive tract ultrasound examinations were performed in 8887 beef females (6355 heifers and 2532 cows) that were artificially inseminated in 12 spring calving herds between 2011 and 2016. Transrectal ultrasonography was performed by one clinician using a real-time, b-mode ultrasound scanner with a 5-MHz transducer (Aloka 500, Sysmed Lab Inc., Chicago, IL or SonoScape S8, Universal Diagnostic Solutions, Oceanside, CA). Only pregnancies between 30 and 120 days were included in this study. The following embryo or fetal parameters unique to specific stages of development were used to estimate the gestational age - an embryo (1 cm wide) with heartbeat by Day 30 of gestation; differentiation of the head and abdominal regions by Day 35 of gestation; budding of the limbs by Day 45 of gestation; crown to rump length, abdominal, thoracic, head (width and/or occipitonasal length), placentome and umbilical cord sizes; amniotic sac and orbital diameters.

## Data management

Only cows bred to Angus sires and cows that calved between 260 and 300 d after fixed time AI (FTAI) were used in the analysis. Gestational age difference was calculated as actual (i.e., days since most recent breeding) minus estimated (i.e., based on estimate from ultrasonography) age of the conceptus. Therefore, an underestimation (positive gestational age difference) means the ultrasound estimated age of the conceptus was younger than the actual gestational age and an overestimation (negative gestational age difference value) means the ultrasound-estimated age of the conceptus was older than the actual gestational age. Projected calving dates for a gestation length of 283 d were calculated for ultrasonography gestational age and actual breeding dates. These dates were compared to actual calf birthdates to determine error in pregnancy diagnosis. The error rate was defined as number of calving over/underestimated (occurred before 278 and after 287 days after FTAI) divided by total number of calving. Sensitivity analysis for the gestation lengths 281 and 285 d were also calculated.

Data including age of the dam, body condition of the dam (1 to 9; 1, emaciated; 9, obese), sire of the calf, sire's calving ease estimated progeny difference (EPD) score, date of insemination, date of ultrasound pregnancy diagnosis, date of calving, number of calves recorded at birth, gender of calf born, calf weight, dystocia in the subsequent calving, and stillbirths were recorded. Age records were categorized as 1, 2, 3 to 6, 6 to 10 and >10. Body condition were categorized as <5, 5 to 7 and >7.

#### Statistical analysis

Data were analyzed with a statistical software program (SAS Version 9.4 for Windows, SAS Institute, Cary, NC). Differences in the mean gestation length were analyzed using ANOVA (PROC GLM of SAS). The Bartlett test was used to assess homogeneity of variance. The data for heifer and cow groups were analyzed separately. Wherever variances for the mean gestation length were heterogeneous,

a log10 transformation was performed. All values are presented with non-transformed values. All pairwise differences between factor level means were analyzed by Tukey's method.

Factors associated to differences in birthweight of calf were determined by ordinal regression analysis. Variables included in the model were calf's gender, dystocia, calving ease estimated progeny difference and age of the dam. Factors associated gestational age differences were determined using a mixed model. Fixed variables included in the model were calf gender, calf body weight, sires calving ease EPD and age of the dam and dam's body condition score. Location clustered with in the year (year [location]) was included as random variables in the model.

Factors associated with prediction accuracy of calving date by ultrasound based gestational age were determined using a mixed model. Fixed variables included in the model were calf gender, calf body weight, sires calving ease EPD score and dams' age. Year (location) offered as random effects. The error rates on the accuracy of calving date prediction for ultrasound and actual gestational ages in heifers and cows were calculated. The error rate was defined as number of calving that occurred outside the gestation length window divided by total number of calving (for gestation length  $283 \pm 7$  days [mean  $\pm 2$  standard deviation {sd}], any calving that occurred < 276 or > 290 days was considered as error in calving prediction for both methods).

## Results

The mean ( $\pm$  SD) gestation length for the study population is 283.68  $\pm$  3.52 days. The frequency histogram for gestation length based on breeding date is given in the figure. Least square mean ( $\pm$  SEM) gestational length for heifers and cows are given in Table 1. The gestation length was different for gender of calf, calves that experienced difficult birth or not, sire's calving ease EPD and parity of cow.

Calf's gender (P<0.0001), incidence of dystocia (P<0.01), sire's calving ease EPD (P<0.0001) and age of the dam (P<0.0001) influenced the mean birth weight of calves (Table 2). The mean ( $\pm$  SEM) birth weight of male and female calves were  $82 \pm 0.22$  and  $78 \pm 0.36$ , respectively. The mean ( $\pm$  SEM) birth weight of calves that experienced dystocia or not were  $86 \pm 0.32$  and  $79.0 \pm 0.28$ , respectively.

The result of multivariate analysis for the effect of calf gender, dystocia, calf birth weight, and dam age on the gestation length is given in Table 3. The calf gender, dystocia, calf birth weight, and dam age affected the gestation length (P<0.01).

The result of multivariate analysis for the effect of calf gender, dystocia, calf birth weight, and dam age on the accuracy of calving date prediction based on ultrasound pregnancy diagnosis is given in Table 4. Age of dam and sire's calving ease EPD score influenced the accuracy of calving prediction (P<0.01).

The differences in the accuracy of calving prediction for both methods is given in Table 5. For  $283 \pm 7$  days gestation length in heifers, the error rate for calving date prediction accuracy by ultrasonography and calving calendar was 22.1 and 9.9% respectively; whereas for similar gestation length in cows, the error rate for calving date prediction accuracy by ultrasonography and calving calendar was 13.5 and 9.1%, respectively. Similarly for  $285 \pm 11$  days gestation length in heifers, the error rate for calving date prediction accuracy by ultrasonography and calving calendar was 18.3 and 8.4%, respectively; whereas for similar gestation length in cows, the error rate for calving date prediction accuracy by ultrasonography and calving calendar was 18.3 and 8.4%, respectively; whereas for similar gestation length in cows, the error rate for calving date prediction accuracy by ultrasonography and calving calendar was 18.3 and 8.4%, respectively; whereas for similar gestation length in cows, the error rate for calving date prediction accuracy by ultrasonography and calving calendar was 18.3 and 8.4%, respectively; whereas for similar gestation length in cows, the error rate for calving date prediction accuracy by ultrasonography and calving calendar was 9.1 and 4.7%, respectively.

In addition, the differences on the accuracy of calving date prediction based on ultrasound pregnancy diagnosis and breeding date for different gestational ages are given in Table 6.

#### Discussion

In this study, errors rates for calving date prediction were evaluated to determine whether producers can rely on breeding date or ultrasonography pregnancy diagnosis. The error rates for calving date prediction was lower for ultrasonography compared to breeding date validated the importance of ultrasonography pregnancy diagnosis.

In this study, gender of the calf, age of dam, calf sire EPD and dystocia influenced the gestational length. The effects of gender of the calf were significant for birth weight, (heifer calf - 76.3 lbs vs. bull calf - 81.2 lbs). Birth weight of calves that were born to dams in 2, 3 to 6 and >6 years age groups were

significantly different, 72.3, 2 80.6 and 82.4 lbs, respectively. Calves born to sires with greater calving ease EPD had lower birth weight. Birth weight for calves that were delivered with assistance was greater (82.3 lbs) compared to calves that were born normally (79.0 lbs). It is plausible that the traits that contributed to increased birth weight may have led to prolonged gestation length.

In this study, the gestational age using ultrasonography was determined using different fetal and maternal parameters. Pregnancies were examined with at least two measurable characteristics defined in the material and methods. Embryo size, fetal size (crown-rump length), placentome sizes, size of the head (width and/or occipitonasal length) of the fetus, and diameters of the abdomen and thorax were visualized more frequently, and the diameter of umbilicus less frequently. Studies that investigated the associations of gestational age and fetal parameters concluded that fetal size provided the most precise estimate of gestational age. Hannum et al showed residual sd + 4.5 days for fetal size,  $\pm$  6.9 to 8.7 days for head length and the diameters of trunk, head and nose and  $\pm$  12.6 days for uterine diameter.<sup>14</sup> In the current study the residual sd was +3.5 days.

The placenta plays a crucial role in the development of the fetus. Placental characteristics such as the weight and volume of the placentomes, sizes including length, height and change in the placentomes density was used to estimate gestational age. During transrectal ultrasonography pregnancy diagnosis, placentomes were first visible around Day 35 of gestation. These can be viewed as flattened, semicircular elevations on the surface of the uterine lumen. Results from studies that evaluated the usefulness of placentome parameters to determine gestational ages, revealed that placentomes sizes are not significantly associated with gestational age during both transrecatal<sup>21</sup> and transabdominal<sup>9</sup> untrasonography. Blankenvoorde claimed that there was no significant effect of the breed and age of the dam or uterine horn (gravid vs. non-gravid) on placentome size (P > 0.05) but observed significant association between gestational age and placentome size (P<0.001).<sup>22</sup> Collectively, significant variations in size of placentomes impedes their use as a criteria for fetal ageing. However, a recent study claimed that the measurement of several placentomes sizes could be used to determine fetal age in late gestation.<sup>23</sup> Others have noted that there is a significant increase in the average placentome length with increasing gestational age should be taken into account while determining fetal age.<sup>23</sup>

There was a strong positive correlation between estimated gestational age from ultrasonography and actual gestational age based on the breeding date consistent with similar studies that used ultrasonography to estimate gestational age in cows.<sup>3,24</sup> Overestimation of the gestational age by ultrasonography was observed in the current study. Fitzgerald et al observed overestimation of the embryonic age and underestimation of the fetal age and suggested that alterations in the conceptus-touterine lumen volume ratio may have contributed to this inconsistency.<sup>24</sup> It is interesting to note that the error rate for ultrasonography in heifers was less for 60 to 90 days and higher for 30 to 60 and 90 to 120 days of gestation; whereas the error rate in cows was higher for 60 to 90 days gestational age and less for 30 to 60 and 90 to 120 days of gestation in the current study. It is possible that a combination of distinct fetal differentiation in older-stage pregnancies along with greater uterine capacity in multiparous cows may interfere with the estimation of gestational age using ultrasonography and may account for some of this prediction error. It should be noted that conceptus-to-uterine lumen volume ratio was not studied in the current study. However when fetal, abdominal and thorax sizes were considered to estimate fetal ages, differences in the uterine volume in relation to position of the fetus may have contributed to the error.

The error rate for calving date prediction for ultrasonography was minimal when pregnancy diagnosis occurred between 60 and 90 days gestational age compared to 30 and 60 or 90 to 120 days gestational ages. Normal intrauterine growth takes place in phases–an embryonic and a fetal phase.<sup>25</sup> The embryonic phase consists of proliferation, organization and differentiation of the embryo, whereas the fetal phase consists of continuing growth and functional maturation of the various tissues and organs. The fetal phase of intrauterine development depends on genetic, placental, and maternal factors. These factors plausibly contribute to the asymmetric fetal growth which occurs in late gestation. It is possible that variation in proliferation, organization and differentiation of the embryonic phase (<60 days gestational age) and asymmetry growth during later fetal phase (>90 days gestational age)

could be contributed to higher error rate compared to transition phase from embryo to fetus (between 60 and 90 days gestational age) with minimal error rate.

In heifers, the error rates were higher than for cows. Kramer et al. concluded that first parity influenced the calving date prediction.<sup>26</sup> Crews Jr. suggested a linear effect of age of dam on gestation in which increasing age of dam was associated with longer gestation<sup>27</sup> consistent with the current study. It should be noted that the size (length and width) of the uterus increase progressively with increasing parity, and therefore, gestational age may be overestimated because of the increased conceptus-to uterine lumen ratio in heifers.

Tactical use of reproductive ultrasound can help enhance the overall productivity of the herd. Given the seasonal nature of the beef farming, the use of ultrasound for reproductive management tools tends to be concentrated during various stages of the production cycle. Pregnancy diagnosis at an early stage of pregnancy provides a tool for many producers, both seedstock and commercial, to identify AI-impregnated versus cleanup bull-sired calves.<sup>28</sup> In addition, earlier detection of non-pregnancy diagnosis assists with management decisions to cull open cows. Further identification of cows that were to become pregnant very late in the breeding season also assists with management decisions to sell them as pregnant animals in order to maintain a short calving season.

This study was conducted to determine the error rates for projected calving date based on ultrasound determined gestational age in comparison to actual gestational age. The results from the study revealed that the ultrasound determination of calving prediction was more accurate than traditional breeding date based calving prediction.

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Age	Effect	n	Gestation length
group			C
Heifer	Gender		
	Bull	3396	$281.11\pm0.66^{\text{a}}$
	Heifer	2959	$283.63 \pm 0.67^{\mathrm{b}}$
	Sire calving ease EPD		
	$\leq$ 5	1067	$282.91\pm0.52^{\rm a}$
	6 to 10	3056	$281.22\pm0.43^{\text{ab}}$
	>10	2232	$280.90 \pm 0.31^{\rm b}$
	Dystocia		
	Yes	181	$284.12\pm0.16^{\mathtt{a}}$
	No	6174	$281.19 \pm 0.73^{\rm b}$
Cow	Gender		
	Bull	2367	$283.23\pm0.52^{\rm a}$
	Heifer	1981	$285.41 \pm 0.56^{\rm a}$
	Sire calving ease EPD		
	$\leq$ 5	563	$281.92 \pm 0.63^{\rm a}$
	6 to 10	1668	$283.67\pm0.34^{\text{b}}$
	>10	2117	$285.23\pm0.54^{\rm c}$
	Age		
	2	832	$281.25\pm0.32^{\rm a}$
	3 to 6	1519	$282.19\pm0.29^{ab}$
	7 to 10	1144	$283.92\pm0.32^{\text{b}}$
	>10	853	$285.55\pm0.39^{\rm c}$
	Dystocia		
	Yes	219	$286.12\pm0.22^{\mathrm{a}}$
	No	4129	$283.94 \pm 0.85^{b}$

Table 1. Gestation length least sc	uares mean $\pm$ SEM for calf	gender, sires' calving	g ease EPD, and dam age and breed
		0 ,	

 Table 2. Effect of calf's gender, dystocia, calving ease estimated progeny difference (EPD) score, and dam's age on birth weight of the calf

Predictor	Coef	SE Coef	Ζ	Р	Odds Ratio	Lower	Upper
						95% CI	95% CI
Constant	7.97635	1.00703	7.92	0			
Calf's gender	-0.86312	0.120359	-7.17	0.0001	0.42	0.33	0.53
Dystocia	1.03038	0.215744	2.48	0.013	1.36	1.16	1.81
Calving ease EPD score	-0.05432	0.010617	-5.12	0.000	0.56	0.33	0.68
Age of dam (yrs)	-0.21567	0.024057	-8.96	0.000	0.81	0.77	0.84

Table 3. Multivariate analysis for the effect of gender of calf, dystocia, calving ease EPD, birth weight of calf, and age of the dam on gestation length.

Predictor	Coef	SE Coef	Z	Р	Odds Ratio	Lower 95% CI	Upper 95% CI
Constant	8.73983	1.09137	8.01	0	-	-	-
Gender of calf	-0.0486	0.116411	-0.42	0.0167	1.95	1.76	2.20
Dystocia	0.372988	0.14422	3.66	0.0142	1.58	1.31	2.96
Calving ease EPD	-0.02125	0.005781	-3.68	0.0001	0.88	0.87	0.89
Age of dam	0.0304	0.022754	3.34	0.0116	1.17	1.11	1.23
Calf birth weight	0.414523	0.161287	4.12	0.0018	1.40	1.22	1.51

Predictor	Coef	SE Coef	Ζ	Р	Odds Ratio	Lower	Upper
						95% CI	95% CI
Constant	3.27163	0.389028	8.41	0	-	-	-
Calf gender	-0.1326	0.207646	-0.64	0.0523	0.88	0.58	1.32
Dystocia	0.298869	0.659154	0.45	0.65	1.35	0.37	4.91
Age of dam (yrs)	0.390264	0.150928	2.59	0.01	1.48	1.1	1.99
Sire calving ease EPD	0.219147	0.05572	3.93	0	1.25	1.12	1.39
Calf birth weight	0.004658	0.040815	0.11	0.909	1.42	0.75	4.87

Table 4. Multivariate analysis for the effect of gender of calf, dystocia, sire calving ease EPD, birth weight of calf, age of the dam on the accuracy of calving date prediction based on ultrasound pregnancy diagnosis.

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Parameter	Heifer	(n=6355)	Cows	(n = 4348)
	Based on AI date and	Based on US gestational	Based on AI date	Based on US gestational
	actual calving	age and actual calving	and actual calving	age and actual calving
# of calves born outside of $281 \pm 7$ (%)	$1248 (19.6)^{***}$	$588(9.3)^{***}$	$413(9.5)^{*}$	278 (6.4)*
# of calves born outside of $283 \pm 7$ (%)	$1404(22.1)^{***}$	$(629 (9.9)^{***})$	585 (13.5)*	395(9.1)*
# of calves born outside of $285 \pm 7$ (%)	$1163(18.3)^{**}$	534 (8.4)**	$395(9.1)^*$	207 (4.7)*
Within heifers and within rows: $* = <0.0$	5; ** = <0.01;			
Within cows and within rows: $** = <0.01$	1; *** = <0.001;			

Table 4. Error rate for calving date prediction for ultrasonography and actual gestational ages

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Group	Embryo or	n	Error rate (%) based on US	Error rate (%) based on
	fetal age @ US		determination of gestational age and	breeding date and actual
			actual calving (n)	calving (n)
Heifer	30 to 45	393	10.9 (43)	24.4 (96)
	46 to 60	1006	10.5 (106)	24.1 (243)
	61 to 75	1914	9.2 (176)	21.3 (407)
	76 to 90	1756	9.7 (170)	21.2 (372)
	91 to 105	874	10.5 (92)	22.4 (196)
	106 to 120	413	10.2 (42)	21.8 (90)
	Total	6355	9.9 (629)	22.1 (1404)
Cow	30 to 45	222	7.2 (16)	15.3 (34)
	46 to 60	721	9.6 (69)	11.8 (85)
	61 to 75	1314	9.7 (128)	14.0 (184)
	76 to 90	1222	9.2 (112)	13.5 (165)
	91 to 105	556	7.7 (43)	13.1 (73)
	106 to 120	313	8.9 (28)	14.7 (46)
	Total	4348	9.1 (396)	13.5 (587)

Table 5. Error rate for calving date prediction for ultrasonography and actual gestational ages at different stages (gestation length  $283\pm7$ ).

Table 6. Error rate (%) due to over or under estimation in gestational ages and calving prediction.

Gestation length	Gestation length Estimation		er	Cow		
		Breeding date	Ultrasound	Breeding date	Ultrasound	
		(n=1404)	(n=629)	(n=585)	(n=395)	
281	Under	70.8 (994)	15.7 (99)	66.3 (388)	29.9 (118)	
	Over	29.2 (410)	84.3 (530)	33.7 (197)	70.1 (277)	
283	Under	67.2 (943)	13.8 (87)	61.4 (359)	28.1 (111)	
	Over	32.8 (461)	86.2 (542)	38.6 (226)	71.9 (284)	
285	Under	69.2 (972)	16.9 (106)	64.8 (256)	26.6 (105)	
	Over	30.8 (432)	83.1 (523)	35.2 (139)	73.4 (290)	



Figure. Histogram of gestation length frequency; mean  $\pm$  SD: 283.68  $\pm$  3.52