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Comparison of Fetal Losses from Diagnosis of Pregnancy Using Ultrasonography or Rectal Palpation in Beef Heifers by Novice or Experienced Technicians

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ABSTRACT

Pregnancy diagnosis is widely practiced in cattle production systems. Ultrasonography is an alternative technique to rectal palpation for pregnancy diagnosis. Fetal losses caused by rectal palpation are well documented; however, reported losses from ultrasonography for pregnancy diagnosis are often confounded by normal embryonic losses during early gestation. Losses caused by inexperienced technicians have been reported previously, but limited information is available on technicians that are in the learning process. Our objectives were to compare fetal losses from pregnancy diagnosis during early gestation for 1) stage of gestation at the time of diagnosis (<53 or ≥53 d), 2) method of diagnosis (ultrasonography or rectal palpation), and 3) different skill levels of the technicians (novice or experienced). Beef heifers (n = 2,190) exposed to natural service for 27 d, followed by diagnosis of pregnancy between 42 and 74 d of gestation were used to evaluate these objectives. Overall loss was 1.55%. Risk of loss was greater ($P < 0.01$) in heifers <53 d pregnant compared with heifers ≥53 d (3.46 vs. 1.26%; a 2.74-fold increase) at the time of evaluation. Greater fetal loss ($P = 0.051$) occurred with rectal palpation than with ultrasonography (2.68 vs. 1.29%; a 2.08-fold increase). Heifers evaluated by inexperienced technicians had a 2.07% fetal loss, whereas heifers evaluated by experienced technicians had only a 1.06% loss ($P < 0.01$; a 1.95-fold difference). Cattle producers and veterinarians should recognize the importance of stage of pregnancy, level of technician experience, and method of diagnosis used to reduce losses attributable to pregnancy diagnosis.

Key words: pregnancy diagnosis, rectal palpation, reproductive loss, technician experience, ultrasonography

INTRODUCTION

The development and breeding of beef replacement heifers is a large investment for the beef producer (Meek et al., 1999). Determination of pregnancy has routinely been incorporated as a tool in replacement heifer management (USDA, 2009). Wisnicky (1948) described a manual method that has become the standard method for assessing pregnancy in cattle. Historically, this method of pregnancy evaluation has been widely practiced in the dairy industry and among beef production systems, especially in the western United States (USDA, 2009). In recent years, the use of ultrasonography for pregnancy determination has been established (Hanzen and Delsaux, 1987; Beal et al., 1992; Fricke, 2002, Lamb and Fricke, 2005). Fetal losses associated with the use of rectal palpation are well documented (Abbitt et al., 1978; Franco et al., 1987). The reported losses associated with the use of ultrasonography for pregnancy diagnosis are often con-

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founded by the effects of normal embryonic losses during early gestation (Beal et al., 1992; Lamb et al., 1997). Studies are lacking on the comparison of reproductive losses associated with the techniques of rectal palpation and ultrasonography without the confounding of normal embryonic losses. Embryonic losses are defined as the normal attrition of embryos that occurs up to approximately 37 to 42 d of gestation (Fosgate and Smith, 1954; Romano et al., 2007). Abbitt et al. (1978) reported losses with clinicians at different skill levels, but limited information is available on technicians that are in the learning process. The objectives of this study were to compare reproductive losses from pregnancy diagnosis during early gestation for 1) stage of gestation at the time of diagnosis (<53 or ≥53 d), 2) method of diagnosis (ultrasonography or rectal palpation), and 3) different skill levels of the technicians.

MATERIALS AND METHODS

The animals used in this study were managed in accordance with best practices for low-stress animal handling and Colorado State University Animal Care and Use guidelines. No more than 3 rectal examinations (palpation or ultrasonography) were conducted on any 1 animal to minimize animal discomfort. Replacement beef heifers (n = 2,190) from 4 herds on the same ranch in Western Nebraska were examined for pregnancy by either ultrasonography or rectal palpation and by experienced or novice technicians.

Heifers were developed during the winter on native Nebraska Sandhills range with minimal harvested feed inputs of meadow hay and with a commercial protein supplement fed as a compressed cake to a prebreeding target BW of approximately 55% of expected mature BW. Routine prebreeding management practices included the collection of individual heifer BW and vaccinations for viral reproductive diseases (Bovishield Gold FP, Pfizer Animal Health, New York, NY) in April 2007, approximately 40 to 50 d ahead of the breeding season. Heifers were all naturally serviced on native Sandhills range for 27 d (June 12 to July 9, 2007). Bulls used for breeding received a reproductive soundness examination in late April according to the guidelines of the Society for Theriogenology (Chenoweth et al., 1992; Beef Improvement Federation, 2006). Only bulls receiving a Satisfactory classification were placed in the breeding pastures. The average heifer-to-bull ratio was 29:1.

Pregnancy was diagnosed by either rectal palpation or ultrasonography from August 20 to 25, 2007; therefore, heifers could have been 42 to 74 d pregnant at the time of diagnosis. An Aloka 500 ultrasound instrument with a 5-MHz linear probe (Aloka, Wallingford, CT) was used for ultrasonography. Evaluations were done by 12 technicians, with 10 technicians having limited experience. The 10 novice technicians were either senior veterinary students or first-year veterinary interns in the food animal medicine program at Colorado State University. Novice technicians had elected to take additional training in beef cattle pregnancy diagnosis as part of their training program. All novice technicians had previously received classroom instruction and, in most cases, had also received some laboratory and field instruction on both pregnancy diagnosis techniques before the initiation of the rotation. The 2 experienced technicians had extensive practical rectal and ultrasound diagnosis training with more than 30,000 palpations and 2,500 ultrasonography evaluations each.

Early in the data collection week, the proper process of pregnancy evaluation by both methods (palpation and ultrasonography) was taught to all novice technicians and verified by an experienced technician. As the week progressed and technical competency of the novice technicians increased, they were allowed to diagnosis pregnancy by either technique on their own or together with other novice technicians. They also were allowed the option of having an experienced technician verify their diagnosis. Thus, individual heifers may have been evaluated by up to 3 inexperienced technicians or an experienced technician. Technicians not only determined the pregnancy status but also the duration of pregnancy. The range of duration of pregnancy at the time of initial evaluation was between 42 and 74 d of gestation. Based on an earlier study (Abbitt et al., 1978) of pregnancy losses, and to establish the most practical information from our study, duration of pregnancy was divided into 2 categories (i.e., <53 or ≥53 d of pregnancy). At 53 d of pregnancy, the amnionic vesicle is clearly distinguishable and can be measured by both rectal palpation and ultrasound techniques. Data were tabulated as novice only or as experienced technician, whether experienced technicians evaluated pregnancy singly or together with novice technicians.

Considerable planning and recording effort was done to ensure the accuracy of data collection. Because multiple heifers could be evaluated simultaneously by different technicians, a chalk-marking system was developed to track the technician, estimated duration of pregnancy, and method of evaluation. On release from the pregnancy testing chute, the heifers entered a hydraulic squeeze chute where BCS (Richards et al., 1986) and BW were recorded.

The design of this experiment had to fit the management system of the ranch and still address the study questions. The ranch managers allowed reevaluation only of heifers that displayed standing estrus 35 to 41 d after the initial pregnancy diagnosis. To accomplish this, heifers received an Estrotect heat detection patch (Estrotech, Spring Valley, WI) on the tail head at the time of the first pregnancy diagnosis. Over the succeeding days, the herd manager periodically sorted heifers from the group that had at least 50% of the patch color rubbed off or that had lost the patch because of repeated mountings. A total of 855 heifers were reevaluated between 77 and 112 d of gestation to establish the level of fetal loss. The reevaluation was done by a single experienced technician by rectal palpation. Because of complexities involved with using calving date to predict actual breeding date, birth date was not used to validate fetal aging at pregnancy evaluation.

Statistical analysis was conducted using MlwiN software (Rasbash et al., 2005), a statistical software package for fitting multilevel models that uses both maximum likelihood estimation and Markov chain Monte Carlo methods. Variables included the herd designation, the method of evaluation (palpation vs. ultrasound), experience level of the technician or technician team (novice vs. experienced), technician(s) (designated A to E, H, J, and X), number of evaluators (1 to 3) per heifer, duration of pregnancy at the time of examination (<53 vs. ≥53 d), number of heifers examined by each detector or detection team for each herd in each pregnancy duration group, and number of heifers later found open of those initially examined. Heifers evaluated by 1 or more novice technicians only were designated as being in the novice group. The experienced evaluator group included heifers evaluated by an experienced individual either alone or together with a novice technician. Logistic regression analysis with random effects was used to identify factors associated with an increased or decreased risk of fetal loss after pregnancy diagnosis while accounting for clustering at the herd and experience level.

Variables were screened for association with pregnancy loss using univariate logistic regression, and were eligible for inclusion in a multivariate model if $P \leq 0.25$ on Wald testing. The foundation for the multivariate logistic regression model was the best fitting (lowest P-value) univariate model. All possible 2-variable models were evaluated, and the best fitting model (lowest P-value) was used as the basis for 3-variable models. This process was continued until inclusion of additional variables no longer improved model fit (Wald P-value >0.05). If not already in the model, diagnosis method was forced into the model to address the a priori hypothesis of the study. All possible 2-way interactions were evaluated and were retained if they significantly improved model fit ($P \leq 0.05$). Herd was evaluated as a potential confounder and was retained if it altered parameter estimates by $>10\%$.

Table 1. Results of univariate analyses of specified variables for their association with early pregnancy loss in beef heifers evaluated by palpation or ultrasonography with novice or experienced evaluators

Variable	Factor	Odds ratio[1]	Wald P-value[2]
Method	Palpation	1	—
	Ultrasonography	0.51	0.07
Experience	Novice	1	—
	Experienced	0.52	0.06
No. of palpators	1	1	—
	2	0.73	0.31
	3	2.58	0.24
Stage of pregnancy	<53 d	1	—
	≥53 d	0.32	<0.01

[1]The odds ratio is a method of comparing whether the probability of a certain event is the same for 2 groups. An odds ratio of 1 implies that the event is equally likely in both groups. An odds ratio greater than 1 implies that the event is more likely in the first group. An odds ratio of less than 1 implies that the event is less likely in the first group.

[2]The Wald test compares specifications of nested models by assessing the significance of restrictions to an extended model with unrestricted parameters.

RESULTS AND DISCUSSION

The univariate analysis individually considered the effect of each variable separately to identify which variables had the greatest impact on pregnancy loss. Univariate analyses identified method of diagnosis, technician experience level, and stage of pregnancy as individual potential risk factors associated with loss of pregnancy attributable to diagnosis (Table 1). When considered as a univariate, the odds ratio for method of diagnosis showed a tendency ($P = 0.07$) for ultrasound to be less likely (odds ratio below 1) to cause pregnancy loss than palpation. Similarly, in the univariate analysis, level of technician experience also showed a tendency ($P = 0.06$) for experienced technicians to be less likely to cause pregnancy loss than novice technicians. Stage of pregnancy at the time of examination had a highly significant ($P < 0.01$) impact on losses, with fetuses less than 53 d of gestation being more susceptible to pregnancy loss than fetuses 53 d or older. There was no difference ($P < 0.24$) in the likelihood of fetal loss attributable to the number of technicians that evaluated a heifer.

Use of the 3 variables identified in the univariate analysis then facilitated determination of the best fitting multivariate model, which included stage of pregnancy, evaluator experience, and method of evaluation (Table 2). Method of diagnosis was forced into the multivariate model despite marginal statistical significance ($P = 0.051$) to accomplish the preplanned comparisons designed in the study. No 2-way interactions significantly improved multivariate model fit. Both herd and number of evaluators were evaluated as potential confounders. They did not alter the estimates of effect for the main variables in the model and were not retained in the model. The final best fit multivariate model is shown in Table 2. The risk of pregnancy loss was lowest ($P < 0.01$) for heifers with a pregnancy ≥53 d and for experienced evaluators. The risk of pregnancy loss was lower when ultrasound methodology was used (P

Table 2. Best fitting model of factors associated with loss of early pregnancy in beef heifers after pregnancy detection by ultrasonography by experienced technicians

Factor	Estimate	SE	Wald P-value[1]	Odds ratio[2]	95% CI
Intercept	−2.37	—	—	—	—
Pregnancy ≥53 d	−1.30	0.40	<0.01	0.27	0.12 to 0.60
Experienced	−1.14	0.42	<0.01	0.32	0.14 to 0.73
Ultrasonography	−0.75	0.41	0.051	0.47	0.21 to 1.05

[1]The Wald test compares specifications of nested models by assessing the significance of restrictions to an extended model with unrestricted parameters.

[2]The odds ratio is a way of comparing whether the probability of a certain event is the same for 2 groups. An odds ratio of 1 implies that the event is equally likely in both groups. An odds ratio greater than 1 implies that the event is more likely in the first group. An odds ratio of less than 1 implies that the event is less likely in the first group. This table indicates the change in odds of pregnancy loss compared with a heifer with pregnancy <53 d examined by a team with novice evaluators using rectal palpation for pregnancy diagnosis.

= 0.051) during the early (42 to 74 d) stage of pregnancy than when rectal palpation was used. There were no significant interactions between the risk factors.

The increased pregnancy loss associated with early-stage pregnancy diagnosis has been reported previously for the palpation method (Abbitt et al., 1978; Paisley et al., 1978; Franco et al., 1987; Alexander et al., 1995). Similarly, increased pregnancy losses have been reported when using ultrasound in the early stages of pregnancy (Beal et al., 1992; Lamb et al., 1997). However, some of the increased losses in pregnancy reported for early detection using ultrasound were confounded by normal embryonic losses and not by method of evaluation. In this study, we minimized the risk of biased interpretation from normal embryonic losses as a confounding factor by assessing a large number of heifers that conceived in a short period of time. The minimal time of evaluation after breeding was 42 d, which is normally beyond the period of time associated with embryonic losses (Fosgate and Smith, 1954; Romano et al., 2007).

Pregnancy losses resulting from apparent trauma during pregnancy diagnosis in this study are presented in Table 3. Overall pregnancy loss in this study was 1.55%, which is consistent with other reports of fetal

Table 3. Number of beef heifers and percentage of early pregnancy when examined by novice or experienced technicians by palpation or ultrasonography at 2 stages of pregnancy

		Stage of pregnancy						Total by method of evaluation		
		<53 d			≥53 d					
Method of evaluation	Experience level	No. of cows	No. open	Loss, %	No. of cows	No. open	Loss, %	No. of cows	No. open	Loss, %
Palpation	Novice	35	2	5.71	476	13	2.73			
	Experienced	194	7	3.61	649	3	0.46			
Subtotal by method		229	9	3.93	1,125	16	1.42	1,354	25	2.68[c]
Ultrasound	Novice	29	1	3.45	523	6	1.15			
	Experienced	31	0	0.00	253	2	0.79			
Subtotal by method		60	1	1.47	776	8	1.11	836	9	1.29[d]
								Total by experience level		
Subtotal by experience level across stage of pregnancy	Novice	64	3	4.69	999	19	1.90	1,063	22	2.07[e]
	Experienced	225	7	3.11	902	5	0.55	1,127	12	1.06[f]
Total by stage of pregnancy		289	10	3.46[a]	1,901	24	1.26[b]			
								Grand total		
								2,190	34	1.55

[a,b]Means within rows differed at $P < 0.01$.

[c,d]Means within columns differed at $P = 0.051$.

[e,f]Means within columns differed at $P < 0.01$.

loss (Whittier, 2009). Risk of pregnancy loss was significantly greater ($P < 0.01$) in heifers at <53 d of gestation compared with heifers at ≥53 d of gestation (3.46 vs. 1.26%) at the time of evaluation. The 2.74-fold increase in pregnancy loss during this early gestation period could be attributed to a greater susceptibility to physical manipulation while the amnionic vesicle and allantochorion membranes of the placenta are developing (Jonker, 2004). A 2.08-fold increase ($P = 0.051$) in pregnancy losses occurred with rectal palpation compared with ultrasonography (2.68 vs. 1.29%). This difference has been stated by others but was not established without the confounding influence of normal embryonic loss. With the shortened breeding season in the heifers, we were able to more appropriately compare the techniques of rectal palpation versus ultrasonography independently of normal embryonic losses. Novice technicians had a 1.95-fold increase ($P < 0.01$) in pregnancy losses compared with experienced technicians (2.07 vs. 1.06%, respectively). This further substantiates the importance of experience in the evaluation of pregnancy regardless of methodology. With the large number of heifers evaluated during this week (n = 2,190), it is possible that misdiagnosis of pregnancy status could have become a factor because of technician fatigue, particularly with rectal palpation. The ability to visualize the pregnancy indicators with ultrasound without considerable palpation effort may have played a role in the lower pregnancy losses found with ultrasonography.

IMPLICATIONS

Regardless of the method of pregnancy evaluation, greater losses should be expected if evaluation of pregnancy occurs between 42 and 52 d of pregnancy. In this study, there was a 2.74-fold increase in losses compared with determination of pregnancy between 53 and 74 d of gestation. Abbitt et al. (1978) previously reported high losses when the pregnancy evaluation was done at less than 52 d of gestation. This study reiterates this observation. Method of diagnosis also affected pregnancy loss, with fewer losses occurring when using ultrasonography. Technicians can cause early fetal death (Alexander et al., 1995). A high level of expertise by rectal palpation can take years to develop, and this does not lend itself to inexperienced technicians building confidence, especially with early diagnosis. Cattle producers and veterinarians should recognize the importance of stage of pregnancy, level of technician experience, and method of diagnosis used to reduce losses attributable to pregnancy diagnosis.

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