Effects of early pregnancy diagnosis by per rectal palpation of the amniotic sac on pregnancy loss in dairy cattle

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Objective—To determine effects of per rectal amniotic sac palpation (ASP) for pregnancy diagnosis during early gestation on pregnancy loss in lactating cows.

Design—Controlled, randomized block design.

Animals—368 pregnant dairy cows.

Procedures—Pregnancy was detected via transrectal ultrasonography (TRUS) at day 29 (day of estrus = day 0), and cows were allocated into a control group (n = 167 cows) and ASP group (180). Control cows were not subjected to pregnancy diagnosis via palpation per rectum. Per rectal ASP was performed between days 34 and 43 by only 1 experienced veterinarian. All cows were reevaluated with TRUS on days 45, 60, and 90.

Results—21 cows were removed because of illness. Pregnancy loss between days 29 and 90 occurred in 44 of 347 (12.7%) cows. Pregnancy loss for the control and ASP groups from days 29 to 90 occurred in 22 of 167 (13.2%) and 22 of 180 (12.2%) cows, respectively. Late embryonic pregnancy loss (days 29 to 45) for the control and ASP groups occurred in 18 (10.8%) and 15 (8.3%) cows, respectively. Early fetal pregnancy loss (days 46 to 60) for the control and ASP groups occurred in 2 of 149 (1.3%) and 6 of 169 (3.6%) cows, respectively, and late fetal pregnancy loss (days 61 to 90) for the same groups occurred in 147 (1.3%) and 6 of 169 (0.6%) cows, respectively.


In cattle, PPR is the most frequent procedure used for pregnancy diagnosis throughout the world.1–4 The intensive use of PPR for pregnancy diagnosis began during the second half of the 20th century.5,6 However, few investigations have been conducted to address important aspects of this procedure, namely safety and accuracy.7,8 New methods of pregnancy diagnosis have been developed or are being developed. Among the newest methods of pregnancy diagnosis are measurement of blood concentrations of early conception factor9,10 and pregnancy-specific proteins11–13 and expression of interferon-τ-stimulated genes.14,15

Nevertheless, PPR remains the method of choice for pregnancy diagnosis in cattle for most veterinarians. Palpation per rectum does not require expensive equipment or submission of samples to a laboratory. In addition, the results are available immediately, which permits veterinarians and farm managers to rapidly make decisions about a particular cow. When performed by experienced veterinarians, it is a highly accurate technique at ≥35 days after breeding. Veterinarians can perform PPR and estimate the age of the conceptus, detect twins, and assess viability of the fetus.1,2,4–7 Additionally, the person performing PPR can obtain important information about the animal, such as body condition score, and assess other internal organs.1,2,16,17

In cattle, pregnancy diagnosis via PPR is based on the detection of at least 1 of the 4 positive signs of pregnancy (ie, detection of the allantochorion, amniotic sac, placentomes, or fetus1,2). However, all of these signs of pregnancy are not detectable simultaneously during pregnancy.1,2 The use of per rectal ASP for early pregnancy diagnosis in cattle was first reported in Germany.5,16 Detection of the amniotic sac during per rectal ASP is used as a positive sign of pregnancy and can also be used to estimate the age of the conceptus during the first 65 to 70 days of gestation.1,16 Furthermore, determining the number of amniotic sacs is requisite for establishing the presence of twins in early gestation.1,2,16

Controversy exists with respect to the effects of PPR for pregnancy diagnosis on pregnancy loss.9,20 The importance of a systematic and nontraumatic technique

ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tr>
<td>ASP</td>
<td>Amniotic sac palpation</td>
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<td>FMS</td>
<td>Fetal membrane slip</td>
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<td>PPR</td>
<td>Palpation per rectum</td>
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<td>TRUS</td>
<td>Transrectal ultrasonography</td>
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for PPR cannot be overemphasized because embryonic or fetal death can be accidentally or iatrogenically induced through PPR.21–24 Rupture of the amniotic sac and crushing of the conceptus via PPR were effectively used to induce abortion of unwanted pregnancies before prostaglandin F2α became commercially available in veterinary practice.21,23,24

Other investigators indicated that ASP caused a pregnancy loss of 6.4% between pregnancy diagnosis at 34 to 50 days after insemination and calving25 and 3.6% between initial pregnancy diagnosis at days 35 to 41 and reexamination at 150 days of gestation.26 In a subsequent study, ASP caused an increase in pregnancy loss, compared with the pregnancy loss for a group of female cattle in which pregnancy was detected on the basis of fluctuation in the gravid uterine horn.27 In that same investigation,27 ASP caused less pregnancy loss than did detection of pregnancy via the FMS technique. Conversely, in 2 other studies,28,29 no differences in pregnancy loss were found between per rectal ASP and the FMS technique. In a later retrospective study30 in which pregnancy in female cattle was diagnosed via ASP between days 30 and 45 after breeding, the pregnancy loss was 19% (as determined by the number of cows that subsequently calved). Finally, several investigations in Germany revealed an association between the use of ASP for pregnancy diagnosis during the embryonic period (first 45 days of gestation),31 especially between 36 and 42 days of gestation, and the risk of atresia coli or atresia jejuni in newborn calves.32,33

All the aforementioned investigations in which per rectal ASP was associated with increases in pregnancy loss had important limitations in the study design. These limitations include lack of a control group that was not palpated per rectum (observational studies), uncertainty of pregnancy status, unknown viability of the embryo or fetus, lack of information on twin conceptuses, combined analysis of data for cows and heifers, multiple examinations of cattle at the same time by more than 1 person, and variable and long intervals between PPR and reevaluation.27–30 These are the same limitations for evaluations conducted to assess the use of the FMS technique for early pregnancy diagnosis.8

The objective of the study reported here was to estimate the effect of per rectal ASP for pregnancy diagnosis during early gestation on pregnancy loss in lactating dairy cows. Our hypothesis was that the proportion of cattle with pregnancy loss during early gestation in a control group of nonpalpated cows would not differ from that for cows in which pregnancy was diagnosed via per rectal ASP.

Materials and Methods

Animals—Lactating cows from the Dairy Cattle Teaching and Research Center of the University of Minnesota were included in the study. Cattle were Holstein, Holstein-Jersey crossbred, and Holstein-Montbéliard crossbred cows. Mean lactation number for all cows was 2.1 (range, 1 to 7). Body condition score (scale of 1 to 5)35 of each cow was assessed at each pregnancy examination. Cows were housed in a tie-stall system. The vaccination protocol for the herd has been reported elsewhere.8 Lactating cows were milked twice each day, and diets were formulated to meet or exceed National Research Council requirements.36 Trace mineral salt and water were provided ad libitum. The study was performed in compliance with established standard operating procedures and guidelines for animal care and use at the University of Minnesota.

Procedures—Each lactating cow was estrus synchronized after a voluntary waiting period of 55 days after parturition. Estrus was synchronized every 2 weeks by use of a controlled internal drug-release device7 that contained 1.38 g of progesterone. A device was placed in the vagina of each cow; 7 days later, the device was removed and prostaglandin F2α (25 mg, IM) was administered. Detection of estrus by visual observation of standing to be mounted was performed for 30 to 45 minutes 4 times/d (1 AM, 7 AM, 2 PM, and 8 PM). Cows were artificially inseminated 6 to 12 hours after initial detection of estrus (day 0 = day of estrus). Cows were inseminated with frozen-thawed semen; semen from several bulls was used for insemination.

Experimental design—Initially, 368 healthy pregnant cows with a viable embryo (as determined via TRUS37 at day 29 (mean, 29.2 days; range, 27 to 36 days) were identified. Cows were allocated into 2 groups (control and ASP groups, n = 184 cows/group) in accordance with a controlled, randomized block design. Pregnant cattle originating from the same estrus synchronization protocol were assigned to blocks prior to allocation to the ASP and control groups.

Cows in the control group were not subjected to additional pregnancy diagnosis via PPR. Cows in the ASP group were subjected to per rectal ASP, which consisted of compression of the gravid uterine horn between the thumb and 2 fingers (middle and index fingers) and detection of the amniotic sac as a small, turgid, slightly oblong, balloon-like structure.1,18 Typically, direct or indirect retraction of the uterine horns was performed prior to ASP. Per rectal ASP was performed once between days 34 and 43 by a board-certified theriogenologist (JR) who had >25 years of experience in bovine practice. When an amniotic sac was identified, the examination was concluded; therefore, it was not possible to detect twin conceptuses. When a cow did not have a positive sign of pregnancy during per rectal ASP, TRUS was immediately performed to confirm the cow was not pregnant. Veterinary students, interns, and residents were not allowed to perform PPR or TRUS on the cows at any time during the experimental period.

All cattle were reevaluated via TRUS at days 45 (mean, 44.9 days; range, 38 to 47 days), 60 (mean, 60.9 days; range, 52 to 71 days), and 90 (mean, 90.4 days; range, 81 to 111 days). Examination on day 45 was used to monitor spontaneous late embryonic death for the control group and the potential immediate deleterious effects of ASP on viability of the fetus. Examination at day 60 was used to monitor spontaneous early fetal death for the control group and the potential late deleterious effects of ASP on viability of the fetus. Examination at day 90 was used to monitor spontaneous late fetal death for the control group and the poten-
tial delayed deleterious effects of ASP on viability of the fetus. Spontaneous pregnancy loss was defined as death or loss of the conceptus without an apparent cause in a healthy cow classified as pregnant on day 29 by use of TRUS.

All TRUS examinations were performed in the morning by the same investigator (JER), who used a machine equipped with a 7.5-MHz linear probe. During TRUS examinations, the investigator removed feces from the rectum of each cow and introduced the probe into the rectum; the investigator avoided grasping or manipulating the uterine horns. A diagnosis of pregnancy loss was made when a heartbeat or signs of degeneration of the conceptus (chorioallantoic membrane, amniotic sac, conceptus, or placentomes) were not detected ultrasonographically or there were ultrasonographic signs of degeneration of the conceptus in a cow previously classified as pregnant during TRUS. Only healthy cows were used in the study. Pregnant cows that developed clinical or subclinical mastitis (California mastitis test score ≥ 3), lameness (≥ 3 on a scale of 1 to 5), or digestive disorders (eg, diarrhea) were excluded from the study and treated appropriately.

Statistical analysis—The sample size required for a 1-sided alternative hypothesis to detect an increase in pregnancy loss of 5% between groups with pregnancy loss of 10% between days 29 and 90, with α = 0.05 and β = 0.10, was 166 cattle/group. The proportion of cows subjected to per rectal ASP that had pregnancy loss was compared with the proportion of control cows with pregnancy loss during each of the 3 periods by use of the χ² test or Fisher exact test, as appropriate. In addition, a binary logistic regression analysis was performed with the main outcome of pregnancy determined via TRUS at day 90. The main effects of number of conceptuses, ASP, and body condition scores were included in the model. Continuous variables (eg, body condition score) were analyzed with Student t tests for independent variables. Values were considered significant at P < 0.05. A software program was used for analysis of all data.

Results

At the time of per rectal ASP (days 34 to 43), 11 cows had no signs of pregnancy, which was detected via TRUS the same day. An additional 21 cows were removed from the study because of various illnesses detected between the initial time of pregnancy diagnosis via TRUS (day 29) and day 90. Thus, there were 167 cows for the control group and 180 cows for the ASP group at the end of the study. In the ASP group, 157 of 180 (87.2%) evaluations for pregnancy diagnosis via per rectal ASP were performed between days 37 and 43 (Figure 1).

Overall pregnancy loss between days 29 and 90 was 44 of 347 (12.7%; Table 1). Overall pregnancy loss for the late embryonic period (days 29 to 43; 33/347 [9.5%]) was significantly (P = 0.001) higher than for the early (days 46 to 60; 8/314 [2.5%]) or late (days 61 to 90; 3/306 [1.0%]) fetal periods. Pregnancy loss between days 29 and 90 did not differ significantly (P = 0.79) between the control and ASP groups (22/167 [13.2%] and 22/180 [12.2%], respectively). Pregnancy loss during the late embryonic period did not differ significantly (P = 0.44) between the control and ASP groups (18/167 [10.8%] and 15/180 [8.3%], respectively). Pregnancy loss during the early fetal period did not differ significantly (P = 0.93) between the control and ASP groups (2/149 [1.3%] and 6/165 [3.6%], respectively, and pregnancy loss during the late fetal period did not differ significantly (P = 0.79) between the control and ASP groups (2/147 [1.4%] and 1/159 [0.6%], respectively. No significant (P = 0.24) differences were detected in pregnancy loss between the early and late fetal periods.

The proportion of cows with twin conceptuses at the initial pregnancy diagnosis via TRUS (day 29) was 26 of 347 (7.5%). Pregnancy loss for the entire period (days 29 to 90) did not differ significantly (P = 0.29) between cows with a single conceptus (39/321 [12.1%]) and cows with twin conceptuses (3/26 [19.2%]).

Mean ± SD body condition score on day 29 did not differ significantly (P = 0.78) between the control and ASP groups (2.7 ± 0.5 and 2.7 ± 0.3, respectively). Mean body condition score for the control group on days 45, 60, and 90 was 2.7 ± 0.4, 2.8 ± 0.5, and 2.9 ± 0.5, respectively. For the ASP group, mean body condition score on day 45, 60, and 90 was 2.7 ± 0.5, 2.8 ± 0.5, and 2.9 ± 0.5, respectively. Pregnancy loss during the late embryonic period did not differ significantly (P = 0.44) between the control and ASP groups (18/167 [10.8%] and 15/180 [8.3%], respectively).

Table 1—Pregnancy loss in lactating dairy cows for which early pregnancy diagnosis was determined via TRUS on day 29 (day 0 = day of estrus).

<table>
<thead>
<tr>
<th>Group</th>
<th>Day 45</th>
<th>Day 60</th>
<th>Day 90</th>
<th>Total</th>
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</thead>
<tbody>
<tr>
<td>Control</td>
<td>18/167 (10.8)</td>
<td>2/149 (1.3)</td>
<td>2/147 (1.4)</td>
<td>22/167 (13.2)</td>
</tr>
<tr>
<td>ASP</td>
<td>15/180 (8.3)</td>
<td>6/165 (3.6)</td>
<td>1/159 (0.6)</td>
<td>22/180 (12.2)</td>
</tr>
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</table>

Values reported are the No. of cows with pregnancy loss/No. of cows examined on that day (percentage). *Pregnancy was diagnosed via TRUS on day 29 (day 0 = day of estrus). Control cows were not subjected to PPR. Per rectal ASP was performed between days 34 and 43. Pregnancy loss was determined via TRUS at days 45, 60, and 90.
Discussion

In the study reported here, per rectal ASP for pregnancy diagnosis was a safe procedure for the conceptuses, compared with results for conceptuses of the control group. This finding is in agreement with 2 reports, in which investigators used the FMS technique for pregnancy diagnosis. The 3 time points (ie, end of the embryonic period [day 45], early fetal period [day 60], and late fetal period [day 90]) were designed to estimate the potential immediate, late, and delayed effects of ASP on pregnancy loss. Pregnancy loss in these 3 periods did not differ significantly between the groups. In 2 observational studies, investigators reported that per rectal ASP performed 34 to 50 days after insemination was associated with a pregnancy loss of 3.6% and 6.4% at 150 days of gestation or calving, respectively. In a subsequent report, ASP increased pregnancy loss, compared with pregnancy loss for detection of uterine fluctuation alone; however, ASP was less detrimental than the FMS technique. In that study, each female cow was examined per rectum by 1 intern plus 1 fourth-year veterinary student. Interestingly, in a different portion of that same study, there was no difference in pregnancy loss among the 3 techniques (ASP, FMS, or detection of only uterine fluctuation) for pregnancy diagnosis when performed by experienced clinicians.

Pregnancy loss rates of 5.1% for ASP and 4.8% for FMS were reported for female cattle examined between days 30 and 44 after breeding. Another study had similar outcomes, with no differences in pregnancy loss between per rectal ASP and FMS detected. In a study in which 2 people used 3 approaches consecutively (per rectum detection of uterine fluctuation alone, FMS, and ASP) in the same cow between days 42 and 46 after breeding, there was an increase in pregnancy loss. However, it is difficult to determine whether a specific technique for pregnancy diagnosis caused the pregnancy loss or whether it was a combination of the use of all 3 procedures. Finally, there was a pregnancy loss of 19% between initial pregnancy diagnosis made via per rectal ASP (performed between days 30 and 45) and calving in another study.

Results of the present study reaffirmed the importance of the experimental design, which included a control group of pregnant cows that was not palpated per rectum, to differentiate effects of per rectal ASP from spontaneous pregnancy loss in the control group. In previous observational studies, it was not possible to determine the effects of PPR when no concurrent nonpalpated control groups were included. The present study was possible because of the availability of TRUS as an accurate and reliable method of pregnancy diagnosis. To our knowledge, there has been no evidence of an association between ultrasonography and congenital anomalies or pregnancy loss. Therefore, TRUS allowed us to differentiate pregnancy loss between the control and ASP groups in the present study.

No changes in body condition score were detected within or between groups throughout the study. Body condition score influences the establishment and maintenance of pregnancy in lactating dairy cattle. Loss of body condition during the late embryonic and early fetal periods is associated with an increase in pregnancy loss. Maintenance of body condition during the study eliminated a potential confounding factor for pregnancy loss.

Per rectal ASP has the potential to damage a conceptus because the amniotic sac is a turgid structure during the early stages of pregnancy; therefore, direct and excessive palpation can cause a major increase in intra-amniotic pressure. The importance of a systematic and nontraumatic technique of ASP cannot be overemphasized because PPR can result in embryonic or fetal death. Moreover, the potential to cause hemorrhage into the amniotic sac because of rupture of the heart or liver in a conceptus as a result of the large size of these organs at this stage of embryogenesis has been described.

In other studies, typical on-farm procedures were not used during pregnancy diagnosis because pregnant cattle were subjected to PPR by >1 person, multiple techniques for pregnancy diagnosis were used by the same individual during the same examination, or several techniques were used by >1 person in the same female animal. The pregnant cattle for several of these studies originated from clinical teaching programs and may have been subjected to more trauma as the result of less-experienced people or more rigorous and extensive internal genital examinations, compared with examinations typically performed by experienced bovine practitioners in private clinical practice. In the present study, the same approach that is routinely used in veterinary practice was performed (ie, each cow was subjected to per rectal ASP once during a single examination performed by 1 experienced veterinarian).

In the present study, some cows in the ASP group had no positive signs of pregnancy at the assigned day of per rectal ASP; these cows were subsequently confirmed as not pregnant via TRUS. This clearly indicated that this pregnancy loss was not attributable to PPR. This finding is in agreement with that reported in other studies conducted on the effects of FMS on pregnancy loss (ie, a certain percentage of female cattle did not have any positive signs of pregnancy at the assigned day of PPR). The possibility that some pregnant cows with a positive result for per rectal ASP but in which the conceptus was already dead or in the initial stages of degeneration, which was undetectable by PPR, was not tested and cannot be ruled out. In a study of spontaneous pregnancy loss in cattle monitored via TRUS and PPR, per rectal ASP revealed persistence of an amniotic sac for several days after TRUS revealed that the conceptus was no longer viable.

In the present study, pregnancy loss was 9.5% during the late embryonic period, which was significantly higher than pregnancy loss during either of the 2 fetal periods. These results are in agreement with those of other studies, in which it was found that pregnancy loss was higher during the embryonic period than during fetal periods. In observational studies,
RUMINANTS

Pregnancy loss rates of 6%, 8.5%, and 7.2% were reported in female cattle examined via PPR at 35 to 45, 35 to 51, and > 50 days of gestation, respectively. In studies in which pregnancy in cattle was determined on the basis of progesterone concentrations in milk or blood, a pregnancy loss rate of 7.2% (days 28 to 73)22 and 28% (days 20 to 90)23 has been reported. The clinical importance of similar outcomes in studies2,20,27,32–38 with different designs and conducted by different investigators is critical. The earlier the diagnosis of pregnancy is made, especially during the embryonic period, the higher the chances that the female will have spontaneous pregnancy loss. Therefore, pregnant female cattle in which pregnancy is diagnosed during the embryonic period will require additional examinations to ensure that nonpregnant cattle (as a result of pregnancy loss) are not retained in the production system. Pregnancy is a dynamic clinical condition, and female cattle can remain pregnant or have loss of a pregnancy. On the other hand, nonpregnancy is a static clinical condition that, because of the current inefficiency of estrus detection, will most likely remain undetected until the nonpregnant female cattle are examined.3

The pregnancy loss for cows with twin conceptuses was almost double the pregnancy loss for cows with a single conceptus. These results were similar to results of 1 study6 and lower than results reported in other studies (pregnancy loss was 2.2,20 2.8,19 and 3.148 times as high and 2.5% higher for twin conceptuses than for single conceptuses). The present findings revealed the importance of stratifying data by the number of embryos, because randomization to reduce or avoid potential confounding attributable to twin conceptuses, which have an inherent high risk of spontaneous pregnancy loss.

In the present study, per rectal ASP for pregnancy diagnosis during early gestation did not increase the proportion of pregnancy losses in lactating dairy cattle. Thus, this is a safe, efficient procedure for pregnancy diagnosis when performed by experienced veterinarians.

References

30. White ME, LaFaunce N, Mohammed HO. Calving outcomes for cows diagnosed pregnant or nonpregnant by per rectum