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

Juan E. Romano, DVM, PhD, DACT, and Melvyn L. Fahning, DVM, PhD, DACT

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 185 (3.6%) cows, respectively, and late fetal pregnancy loss (days 61 to 90) for the same groups occurred in 2 of 147 (1.4%) and 1 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 factor\(^9,10\) and pregnancy-specific proteins\(^11-13\) and expression of interferons and interferon–γ–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 ≥25 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 fetus\(^1,2\)). 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.\(^5,18\) 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.\(^1,19\) Furthermore, determining the number of amniotic sacs is requisite for establishing the presence of twins in early gestation.\(^1,2,18\) 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...
The vaccination protocol for the herd has been reported elsewhere. Lactating cows were milked twice each day, and diets were formulated to meet or exceed National Research Council requirements. 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 device 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 F$_2$α (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 TRUS) 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. 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 (JER) 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 pregnancy (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 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 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.

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**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 45; 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.

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**Table 1**—Pregnancy loss in lactating dairy cows for which early pregnancy diagnosis was determined via TRUS alone (control group) and via TRUS and per rectal ASP.

<table>
<thead>
<tr>
<th>Group</th>
<th>Day 45</th>
<th>Day 60</th>
<th>Day 90</th>
<th>Total</th>
</tr>
</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>15/160 (9.4)</td>
<td>6/165 (3.6)</td>
<td>1/159 (0.6)</td>
<td>22/180 (12.2)</td>
</tr>
</tbody>
</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.
days 45, 60, and 90 was $2.8 \pm 0.4$, $2.8 \pm 0.5$, and $2.9 \pm 0.5$, respectively. Body condition scores did not differ significantly between the control and ASP groups on days 45 ($P = 0.45$), 60 ($P = 0.86$), and 90 ($P = 0.94$).

**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 non-palpated 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.

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, ascertainment of pregnancy loss was performed by 1 experienced veterinarian.
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 75)35 and 28% (days 20 to 90)36 has been reported. The clinical importance of similar outcomes in studies8,20,27,32–35 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 study8 and lower than results reported in other studies (pregnancy loss was 2.2,20 2.8,35 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 before 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