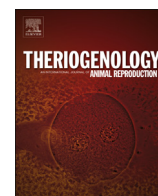




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Effect of early pregnancy diagnosis by per rectum amniotic sac palpation on pregnancy loss, calving rates, and abnormalities in newborn dairy calves



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ARTICLE INFO

Article history:

Received 12 June 2015

Received in revised form 28 August 2015

Accepted 3 September 2015

Keywords:

Cattle
Pregnancy diagnosis
Amniotic sac
Per rectum palpation
Pregnancy loss
Atresia coli

ABSTRACT

The objectives of the present study were to evaluate the effect of per rectal amniotic sac palpation (ASP) for pregnancy diagnosis during the late embryonic period on pregnancy loss, calving rates, and abnormalities in newborn calves. A controlled, randomized, blocked, blind experiment containing 680 lactating pregnant dairy cows with a viable embryo diagnosed by transrectal ultrasonography was performed. Two dairy operation sites (farm A and farm B) were selected. At each farm, the cows were randomly divided into control (CON) and ASP groups. The CON group was not subjected to pregnancy diagnosis via per rectum palpation. The ASP examinations were performed by one experienced veterinarian between Days 34 and 45 after breeding. All cows were reevaluated by transrectal ultrasonography only between 2 and 4 weeks later. Two calving rates were calculated: calving rate 1 (cows that calved from the initial number of pregnant cows) and calving rate 2 (cows that calved from cows pregnant at reexamination). In farm A, the percentages of early pregnancy loss were 11.5% (19 of 165) and 13.2% (24 of 182) for the CON and the ASP groups, respectively ($P = 0.64$). In farm B, the percentage of early pregnancy loss was 11.2% (19 of 170) for the CON group and 8.8% (14 of 159; $P = 0.48$) for the ASP group. In farm A, the percentage of late pregnancy loss was 7.6% (11 of 145) for the CON group and 5.5% (8 of 155; $P = 0.39$) for the ASP group. In farm B, the percentage of late pregnancy loss was 3.7% (5 of 137) for the CON group and 6.3% (8 of 127; $P = 0.32$) for the ASP group. In farm A, early pregnancy loss was higher than late pregnancy loss (12.4% vs. 6.3%; $P = 0.01$), and in farm B, the same tendency was detected (10.0% vs. 4.9%, for early and late pregnancy loss, respectively; $P = 0.02$). In farm A, calving rate 1 was 81.2% (134 of 165) for the CON group and 80.8% (147 of 182; $P = 0.92$) for the ASP group. Calving rate 2 for the same groups was 92.4% (134 of 145) and 94.8% (147 of 155), respectively ($P = 0.68$). In farm B, calving rate 1 was 77.7% (132 of 170) for the CON group and 74.8% (119 of 159; $P = 0.55$) for the ASP group. Calving rates 2 for the same groups were 87.4% (132 of 151) and 82.1% (119 of 145), respectively ($P = 0.20$). Two female calves with atresia coli were

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diagnosed only in the CON group. It was concluded that ASP during the late embryonic period for pregnancy diagnosis did not increase the pregnancy loss, affect calving rates, or produce abnormalities in calves.

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1. Introduction

Per rectum palpation (PRP) is the most frequent procedure used by veterinarians around the world for pregnancy diagnosis in cattle [1–4]. This technique began its usage after the second half of the past century [5,6]. Despite its intensive application, few investigations were designed to answer two important aspects of this practice such as safety and accuracy [7–10].

At present, new methods of pregnancy diagnosis are available in the process of development in cattle [9,11–13]. However, PRP continues to be the procedure of choice for veterinarians for pregnancy diagnosis for several reasons: It does not require equipment or a laboratory, the results are almost immediate, allowing for a rapid decision, and it is an accurate technique after Day 35 of breeding when performed by trained veterinarians [1–4]. Per rectum palpation allows aging of the pregnancy, assesses the viability of the fetus, and has a low cost compared with other procedures [1–4,7,8]. It also gives additional information about other internal organs while simultaneously permitting the examiner to evaluate the body condition score, cleaning score, leg conformation, udder, and other variables [8,14].

Pregnancy diagnosis by PRP is based on the detection of at least one of the four positive signs of pregnancy: allantochorion membrane, amniotic vesicle, placentomes, and fetus [1]. Not all of these signs appear simultaneously during pregnancy [1,3,15]. During earlier stages of gestation, the detection of either the amniotic sac or the allantochorion membrane (also known as fetal membrane slip technique) per rectum is used as a positive sign of pregnancy [16,17]. In addition, the size of the amniotic sac in relationship to the fingers or size of the hand allows one to estimate the age of pregnancy during the first 65 to 70 days of gestation [15]. Moreover, for the diagnosis of twin pregnancies, the identification of the number of amniotic vesicles by PRP is required [1,3,18].

In regard to the effect of PRP for pregnancy diagnosis on the conceptus, conflicting evidence has been published [8,19,20]. Investigators in some studies suggested that PRP had little or no effect on pregnancy loss [21–23]. Conversely, other reports suggested that PRP during early gestation increased pregnancy loss [24–29]. However, these studies had important limitations in their design as previously reported [8]. In recent multiple independent studies, it was shown that the detection of either the allantochorion sac or the amniotic sac by PRP through the embryonic period did not increase the pregnancy loss when reexamined by transrectal ultrasonography (TRUS) in the course of the fetal period [8,19,20]. However, in those studies, no information about calving rates or clinical abnormalities of the newborn calves was reported. Studies in the United States and in other areas of the world have observed an association between amniotic sac palpation

(ASP) during the embryonic period for pregnancy diagnosis until Day 45 of gestation [30] and an increased risk of atresia coli and/or jejuni in newborn calves [31–35]. In atresia coli and/or jejuni, a section of the large bowel or jejuni is absent, resulting in a blind-ending intestinal tube. This clinical congenital condition is lethal, and surgical correction is the only treatment available [36–39]. Atresia coli and/or jejuni has been reported in different countries and in more than 10 breeds of cattle with a marked predominance in Holstein calves [40]. On the basis of those findings, some sources have recommended avoiding PRP of the uterus during the first 45 days of gestation [31–35,41,42]. In spite of this, intestinal atresia was also reported to be inherited as an autosomal recessive trait in Jersey and Swedish Highland cattle [43,44]. Intestinal atresia could develop either from imperfect canalization of the gut or from insufficient blood supply to the affected portion of the intestine [45], and ASP was suggested to act for this last mechanism [33,40]. Nevertheless, the cause of atresia coli and/or jejuni remains controversial and not completely understood [37,40,45]. In general, in the authors' practice, the ASP for early pregnancy diagnosis is not routinely used; however, cases of atresia coli or jejuni were detected. Interestingly, atresia coli and/or jejuni was also diagnosed in newborn calves from dams that underwent PRP only by detection of the allantochorion membrane during the first trimester of gestation either during the late embryonic period or during the fetal period; during the second trimester of gestation, females were diagnosed as pregnant only by TRUS (Romano, unpublished data). These observational findings strongly suggest that ASP during gestation was not associated with this pathologic condition. Unfortunately, at the present time, no controlled randomized studies are available to demonstrate whether ASP for pregnancy diagnosis could produce atresia coli and/or jejuni.

In general, the bovine practitioner performs an early pregnancy diagnosis during the late embryonic period (≤ 45 days) for technical and economic reasons; therefore, accurate information about potential deleterious effect of PRP on conceptus is capital [8]. Transrectal ultrasonography permits an early and accurate method for early pregnancy diagnosis that does not affect the embryo or fetus [11,46–50]. Therefore, the use of TRUS could reduce or eliminate the PRP of the uterus by constructing a better experimental design by creating a contemporaneous control (CON) group of pregnant females that does not undergo PRP that could be contrasted with a treatment group of pregnant females that undergoes PRP. Consequently, the conclusion using this experimental approach will be better and less biased.

The objectives of the present study were to evaluate the effect of ASP for pregnancy diagnosis in lactating dairy cows during the late embryonic period on pregnancy loss, calving rates, and abnormalities in newborn calves.

2. Materials and methods

2.1. Study population

This study was performed at two dairy operation sites; one located in Minnesota and the other in the Texas Panhandle. Lactating cows from the Dairy Cattle Teaching and Research Center of the University of Minnesota at St. Paul, Minnesota (farm A) and from a certified organic dairy farm at Stratford, Texas (farm B), were included. This study was performed in compliance with the established standard operating procedures and guidelines for animal care and use at the University of Minnesota and Texas A&M University. In farm A, cattle were Holstein, Holstein–Jersey crossbred, and Holstein–Montbéliard crossbred animals. In farm B, the lactating females were Holstein, Holstein–Jersey crossbred, and Jersey breed. The average lactation number for all the cows of farm A was 2.1 (range: 1–7) and was found to be 2.5 (range: 1–9) for farm B. Body condition scores were assessed at the time of initial pregnancy diagnosis, and the scale used was from 1 to 5 [51]. The voluntary waiting period in both farms was between 45 and 55 days after parturition. At farm A, estrus was synchronized every 2 weeks by use of a controlled internal drug release device (1.38 g of progesterone; Zoetis Animal Health, Kalamazoo, MI, USA). This device was placed in the vagina of a cow for 7 days and was followed by intramuscular administration of 25 mg of natural PGF₂ α (Lutalyse; Zoetis Animal Health) at the time the CIDR product was used as previously described [52]. Detection of estrus by visual observation of standing to be mounted was performed four times each day at the farm (1 AM, 7 AM, 2 PM, and 8 PM). Detection of estrus was performed for 30 to 45 minutes for each time point. Artificial insemination was performed 6 to 12 hours after initial detection of estrus (Day 0 = day of detection of estrus). In farm B, no estrous synchronization protocols were used. Cows were subject to a reproductive program based on artificial insemination from visual estrus detection. The cows' tail heads were painted daily with colored chalk and checked for estrus by removal of tail chalk. If estrus was determined, cows were artificially inseminated during the morning. In both farms, cows were inseminated with frozen–thawed semen from different bulls. At farm A, lactating cows were housed in a tie-stall system, and in farm B, lactating cows were housed in a free-stall system. During the grazing season (May–September), cows at farm B had access to pasture and grazing which provided a significant portion of the total ration. The vaccination protocol for farm A has already been reported elsewhere [20]. The vaccination protocol for farm B was scheduled at the following periods: 25 to 30 days postpartum with *Escherichia coli* bacterin (Enviracor J-5 *E. coli* Bacterin; Zoetis Animal Health), infectious bovine rhinotracheitis, bovine viral diarrhea types 1 and 2, parainfluenza 3, bovine respiratory syncytial virus, *Campylobacter fetus* and *Leptospira canicola*, *L. grippotyphosa*, *L. hardjo*, *L. icterohaemorrhagiae*, *L. pomona*, and *L. borgpetersenii* (Bovi-Shield Gold FP 5 L5 HB; Zoetis Animal Health). At pregnancy confirmation, a *Lepstopira* bacterin containing *L. canicola*, *L. grippotyphosa*, *L. hardjo*, *L. icterohaemorrhagiae*, *L. pomona* (Leptoferm 5;

Zoetis Animal Health) was administered. At dry-off (–60 days), they were vaccinated with *Clostridium chauvoei*, *C. septicum*, *C. haemolyticum*, *C. novyi*, *C. sordelli-perfringens* types C and D bacterin-toxoid vaccine (Ultrabac 8; Zoetis Animal Health), *E. coli* bacterin again (Enviracor J-5 *E. coli* Bacterin; Zoetis Animal Health), and rotavirus (serotypes G6 and G10), bovine coronavirus, and enterotoxigenic strains of *E. coli* vaccine (ScourGuard 4K; Zoetis Animal Health). At parturition (–15 days), another booster of *E. coli* bacterin (Enviracor J-5 *E. coli* Bacterin; Zoetis Animal Health) was administered.

Lactating cows were milked twice daily in farm A and three times daily in farm B. In both farms, diets were formulated to meet or exceed National Research Council requirements [53]. Trace mineral salt and water were provided ad libitum. Pregnant cows that developed clinical or subclinical mastitis (California mastitis test, ≥ 3), lameness (≥ 3 on a scale of 1–5; [54]), or digestive disorders (i.e., diarrhea) from the date of pregnancy diagnosis to the end of the study were treated accordingly.

2.2. Experimental design

The present investigation used a randomized, controlled, blocked, double-blind design. The blocks for two farms (farms A and B) were used. From each farm, the females were diagnosed as pregnant on the basis of the presence of a viable embryo by TRUS between Days 28 and 45 after breeding [12]. In farm A, the average time for pregnancy detection was 29.2 ± 1.7 days (range: 28–36 days), and for farm B, it was 40.2 ± 3.0 days (range: 35–45 days). All the initial TRUS examinations were performed by the same veterinarian in the morning using a portable ultrasound machine equipped with a 7.5-MHz linear transducer as previously described [20]. Then, the pregnant females were randomly assigned to the CON (no PRP) and treatment groups (ASP group, per rectum ASP). The CON group did not receive any PRP of the uterus. Amniotic sac palpation consists of the compression of the pregnant uterine horn and detection of the amniotic sac as a small, turgid, slightly oblong, balloon-like structure between the thumb and the fingers. All the ASPs were performed only once between Days 34 and 45 after artificial insemination by a board-certified theriogenologist with more than 30 years of bovine experience. If one amniotic sac was found, no attempt was made to find a second one; therefore, no diagnosis of twin pregnancy was done. In general, the uterine horns were retracted directly or indirectly before this approach was used. No veterinary students, interns, residents, or any persons other than the ones involved in the present project were allowed to perform PRP or TRUS on these cows at any time during the experimental period. After being submitted to their respective treatments, each female was reevaluated for pregnancy again between 2 and 4 weeks later only by TRUS in both farms. In farm A, the same veterinarian was involved in the initial treatment and reexamination; however, he was blind to the treatment of each cow at the time of reexamination. Some of these cows were used in a previous study [19]. In farm B, the same veterinarian from farm A was engaged only in the initial pregnancy diagnosis. Pregnancy

reexamination was performed by two different veterinarians who were blind to the treatment but were aware of the project. All of the pregnant females at reexamination were followed until calving. A diagnosis of pregnancy loss was made when a heartbeat or sign of pregnancy (allantochoirion membrane, amniotic sac, conceptus, or placentomes) was not seen by ultrasonography or when signs of conceptus degeneration were observed by TRUS [55]. Every aborted, premature, or stillbirth calf was submitted for necropsy to determine whether abnormalities were present and also to determine the type of abnormality. In farm A, the samples were submitted to the Minnesota Diagnostic Laboratory. In farm B, two independent veterinarians were in charge of these evaluations and were blinded from the initial intervention of pregnancy diagnosis; they were also different from those two veterinarians involved in the pregnancy reexaminations. All the calves born alive were maintained for observation for 3 to 5 days postpartum to detect any type of abnormalities.

Early pregnancy loss was defined as the number of cows with pregnancy loss at reexamination over the number of initially pregnant cows, expressed as a percentage. This is a measure of pregnancy loss from the late embryonic and early fetal periods. Late pregnancy loss was the percentage between the number of cows with pregnancy loss from reexamination to calving and the total number of pregnant cows at reexamination. This is a measure of prenatal pregnancy loss that included only the fetal period from the middle of the first trimester to the last trimester of gestation. Calving rate 1 was defined as the percentage of cows that calved from the initial number of pregnant cows. Calving rate 2 was defined as the percentage of cows that calved from the total cows pregnant at reexamination.

2.3. Statistical analysis

The null hypothesis was that the proportion of pregnancy loss at reexamination, calving rates, and abnormalities in the newborn calves were not different for cows that had ASP or TRUS performed for pregnancy diagnosis during the late embryonic period. The number of cows required for detecting a difference of 10% between groups assuming a pregnancy loss of 12% between initial pregnancy diagnosis and reexamination using an alpha error of 5% and a beta error of 10% (power, 90%) was 295 pregnant animals per group [56,57]. The proportion of pregnancy loss for cattle subjected to ASP was compared with the proportion for CON during the two periods (at reexamination and calving) by use of χ^2 analysis or Fisher's Exact Test as appropriate [56–58]. The continuous variables (lactation number and body condition score) were analyzed by a Student *t* test for independent samples [58]. Cows that were missing at reexamination or at calving were not included in the statistical analysis. All data were expressed as mean \pm 1 standard deviation. Values of $P \leq 0.05$ were considered significant. A software program was used to analyze all data sets [57].

3. Results

At the end of the study, four cows from dairy farm A and 36 cows from dairy farm B between the initial time of

pregnancy diagnosis and the end of the study were gone because they were either sold or dead. The animals were sold for low milk production level, chronic mastitis, conformation, and other internal requirements. In farm B, four cows at reexamination, two from the ASP group, and two from the CON group were missing. The culling rates between the farms (farm A: 1.2% vs. farm B: 10.2%) were different ($P = 0.0001$).

In farm A, the body condition score at the initial pregnancy diagnosis for cows in the CON and ASP groups was 2.7 ± 0.5 and 2.7 ± 0.3 , respectively ($P = 0.78$). In farm B, the body condition score at the initial pregnancy diagnosis for animals in the CON and ASP groups was 2.5 ± 0.2 and 2.6 ± 0.2 , respectively ($P = 0.51$). In farm A, the per rectum diagnosis for the ASP group was initiated at 39.5 ± 1.9 days (range: 34–44 days). The reexamination by TRUS was initiated at 61.1 ± 4.4 days for the CON group and at 60.8 ± 4.2 days ($P = 0.54$) for the ASP group.

In farm B, the per rectum diagnosis for the ASP group was initiated at 40.4 ± 3.0 days (range: 35–45 days). The reexamination by TRUS was initiated at 56.3 ± 9.0 days for the CON group and at 55.3 ± 4.8 days ($P = 0.50$) for the ASP group. In farm A, ASP was performed between Days 34 and 42 in 175 of 181 calves (97%), and in farm B, 110 of 158 calves (70%) underwent ASP between Days 35 and 42.

In farm A, the early pregnancy loss was 11.5% (19 of 165) for the CON group and 13.2% (24 of 182; $P = 0.64$; Table 1) for the ASP group. In farm B, the early pregnancy loss was 11.2% (19 of 170) for the CON group and 8.8% (14 of 159; $P = 0.48$; Table 2) for the ASP group. The early pregnancy loss between farms A (12.4%; 43 of 347) and B (10.0%; 33 of 329) was not different ($P = 0.33$). In farm A, the late pregnancy loss was 7.6% (11 of 145) for the CON group and 5.2% (8 of 155; $P = 0.39$) for the ASP group. In farm B, the late pregnancy loss was 3.7% (5 of 137) for the CON group, and it was 6.3% (8 of 127; $P = 0.32$) for the ASP group. The late pregnancy loss between farms A (6.3%; 19 of 300) and B (4.9%; 13 of 264) was not different ($P = 0.47$). In farm A, the early pregnancy loss was higher than the late pregnancy loss rate (12.4% vs. 6.3%; $P = 0.01$). In farm B, the same results were detected (10.0% vs. 4.9%; $P = 0.02$). In both the farms, the overall early pregnancy loss was higher (11.2%; 76 of 676) than the late pregnancy loss (5.7%; 32 of 564; $P = 0.0005$).

In farm A, calving rate 1 was 81.2% (134 of 165) for the CON group and 80.8% (147 of 182; $P = 0.92$) for the ASP group; calving rate 2 for the same groups was 92.4% (134 of 145) and 94.8% (147 of 155), respectively ($P = 0.68$). In farm B, calving rate 1 was 77.7% (132 of 170) for the CON group and 74.8% (119 of 159; $P = 0.55$) for the ASP group; calving rate 2 for the same groups was 87.4% (132 of 151) and 82.1% (119 of 145), respectively ($P = 0.20$). The calving rate 1 was 80.9% (281 of 347) for farm A and 75.4% (251 of 333; $P = 0.14$) for farm B; calving rate 2 was 92.4% (281 of 304) for farm A and 84.8% (251 of 296; $P = 0.0005$) for farm B. Two calves with atresia coli were diagnosed by necropsy only in the CON group in farm A ($P = 0.23$). These calves were singleton females born alive from Holstein dams and sires. The gestation length was 273 and 288 days. All the fetuses or calves born dead and necropsied were negative for intestinal atresia.

Table 1

Early pregnancy loss, late pregnancy loss, and calving rates 1 and 2 for the amniotic sac palpation (ASP) and the control group (CON) in lactating dairy cows examined by per rectum palpation during late embryonic period in Farm A.

Group	Initial number of pregnant cows	Number of cows with pregnancy loss at reexamination (%)	Number of pregnant cows at reexamination	Missing pregnant cows from reexamination to calving	Number of cows with pregnancy loss between reexamination and calving (%)	Cows that calved	Calving rate 1 (%)	Calving rate 2 (%)
ASP	182	24 (13.2) ^a	158	3	8 (5.2) ^a	147	80.8 ^a	94.8 ^a
CON	165	19 (11.5) ^a	146	1	11 (7.6) ^a	134	81.2 ^a	92.4 ^a
Both groups	347	43 (12.4) ¹	304	4	19 (6.3) ²	281	80.9	93.6

Calving rate 1 = Percentage of cows that calved over the initial number of cows pregnant.

Calving rate 2 = Percentage of cows that calved over the initial number of cows pregnant at reexamination.

^aColumns with the same superscript letters were not significantly different ($P \geq 0.05$).

^{1,2}Rows with the different superscript numbers were significantly different ($P = 0.01$).

4. Discussion

The present results from these two dairy operations confirmed previous findings that ASP during the late embryonic period did not increase pregnancy loss when compared with a contemporaneous CON group of pregnant females not subjected to PRP [19]. The experimental design was an important instrument that allowed for differentiation between spontaneous pregnancy loss of the CON group and the potential effects of the ASP group as previously reported [8,20]. This approach was only possible because TRUS permits an immediate, early, and accurate method of pregnancy diagnosis without any deleterious effect reported on the conceptus [8,46–50]. Therefore, a robust cluster of CON pregnant females for contrast was created facilitating a sound and unbiased comparison with the ASP group [12,56].

Amniotic sac palpation has the potential to damage the conceptus at this embryonic period because the amniotic sac is a turgid structure during this stage of organogenesis; consequently, direct or excessive PRP needs to be avoided in order not to increase the intra-amniotic pressure and consequently rupture the heart or liver resulting in embryo death [1,59–62]. Therefore, a gentle per rectum amniotic sac manipulation for pregnancy diagnosis is essential [1,19].

In this study, the rate of pregnancy loss was high during the late embryonic period compared with later gestational periods, and these outcomes are in agreement with former reports [20,55,63]. The percentage of late embryonic pregnancy loss was doubled compared with fetal

pregnancy loss and in agreement with past studies [8,19,20]. The possible reasons are multiple [55]. Consequently, a female diagnosed as pregnant during early gestation will require further reexamination to reduce the chance of maintaining an undetected nonpregnant female in the production system [8,55].

In this investigation, the entire ASP was performed by only one veterinarian, eliminating the confounding factor of variability among examiners as reported in a former study [24]. In addition, the same technique used regularly in veterinary practice was executed (i.e., each cow had its ASP examined only once by one experienced veterinarian). In previous reports, realistic conditions were not followed during the pregnancy diagnosis procedure because pregnant females underwent PRP sequentially by more than one person, different methods were used at the same time, or different methods were used in the same female by more than one person [8,20,24–27].

Previous studies analyzed the effect of PRP during the late embryonic period on pregnancy loss by two or three reexaminations during the late embryonic and early fetal periods to estimate the potential immediate or late effect of the technique [8,19,20]. However, in those studies, no report of the long-term effect between PRP and the calving rate was presented, and the few studies that informed this outcome did not have a CON group of pregnant females not subjected to PRP for comparison [25–28,64,65]. In the present study, differences were detected between both dairy operations in calving rate 2. The main reason for this disparity between farms was that in farm B, more pregnant

Table 2

Early pregnancy loss, late pregnancy loss, and calving rates 1 and 2 for the amniotic sac palpation (ASP) group in which lactating dairy cows examined by per rectum palpation during late embryonic period in Farm B.

Group	Initial number of pregnant cows	Number of cows with pregnancy loss at reexamination (%)	Number of pregnant cows after reexamination	Missing pregnant cows from reexamination to calving	Number of cows with pregnancy loss between reexamination and calving (%)	Cows that calved	Calving rate 1 (%)	Calving rate 2 (%)
ASP	161	14 (8.8) ^a	145	18	8 (6.3) ^a	119	74.8 ^a	82.1 ^a
CON	172	19 (11.2) ^a	151	14	5 (3.7) ^a	132	77.7 ^a	87.4 ^a
Both groups	333	33 (10.0) ¹	296	32	13 (4.9) ²	251	76.3	84.8

Calving rate 1 = Percentage of cows that calved over the initial number of cows pregnant.

Calving rate 2 = Percentage of cows that calved over the initial number of cows pregnant at reexamination.

^aColumns with the same superscript letters were not significantly different ($P \geq 0.05$).

^{1,2}Rows with the different superscript numbers were significantly different ($P = 0.02$).

lactating cows were removed from the productive herd; around 10% of the lactating pregnant cows at reexamination were absent at calving time. One probable reason was that each pregnant lactating female requires a minimum milk production per day to be maintained in the herd. If these pregnant females were kept in the dairy operation, most likely, no differences between farms would have been detected. The present investigation adds new information suggesting that ASP does not influence the calving rates and extending knowledge about the subject.

It has been reported that early pregnancy diagnosis by ASP during the embryonic period (first 45 days of gestation) [30], especially between 36 and 42 days of gestation, was associated with an increased risk of atresia coli and/or jejuni [31–35]. In the United States, one calf was diagnosed with atresia coli from an investigation with the use of organic phosphate systemic insecticides pour-on [31]; however, these authors mentioned that this abnormality was previously noticed in eight calves from 798 neonatal necropsies covering 15 years from 1963 to 1977 in an experimental station in Montana [41]. A German study reported an increased frequency of clinical cases of atresia coli since 1974, and the prevalence varied from 2% to 22% in different dairy operations [66]. In a further study, from the 524 cows evaluated at less than 40 days of gestation, 28 calves (5.3%; range: 3.2%–9.25%) were born with atresia coli and no cases were reported from 995 cows examined after Day 40 [32]. Müller et al. [33] reported six calves (4.8%) with atresia coli from 125 cows that underwent per rectum palpation at less than 42 days of gestation compared with no cases from 103 females examined after Day 43. In the United States, when PRP was performed between Days 36 and 37 in 198 pregnant females, 10 calves (5.2%) with atresia coli were diagnosed [67]. In a different publication from the same herd, eight affected calves (2.5%) from 327 cows evaluated were reported, whereas pregnancy diagnosis performed after Day 40 corresponded to 359 normal and one affected calf [68]. Finally, in Israel, from 682 pregnant females that underwent ASP at 42 days or less, 47 calves with atresia (6.9%; coli and ilei) were diagnosed; meanwhile, no calves were detected with atresia from 800 pregnant females assessed after Day 43 [35]. The major limitation in all these previous reports was that the source of information was based in observational findings because none of these studies included a comparison CON group of contemporaneous pregnant cows of the same gestational age that did not undergo PRP. Therefore, PRP was a potential confounding factor rather a causative effect because all the females were per rectum palpated.

In the present study, the two calves with atresia coli were diagnosed only in the CON group which is in contrast with all the previous studies that associated ASP during the late embryonic period with atresia coli or atresia jejuni [31–34]. The ASP group included 341 pregnant females that underwent PRP between Days 34 and 45, from which 285 were assessed between Days 35 and 42 of pregnancy, which is the period of highest risk of producing atresia coli. On the basis of the prevalence of atresia coli from previous studies, it was expected that there would be between nine and 32 calves with atresia coli in the present study; however, no cases of atresia coli were produced. This is strong

evidence against the deleterious effect of ASP on the conceptus. On the other hand, the incidence (0.6%) in the present CON group was in agreement with an observational study from a Holstein herd that reported 18 cases of atresia coli (0.76%, range between years from 0% to 1.55%) from 2367 births (from 1974 through 1983) [67]. In another report from the same herd, one calf with atresia coli (0.3%) was detected from 295 normal calves diagnosed from a contemporaneous random mating group when pregnancy diagnosis was performed before Day 40 [68]. To the best of the authors' knowledge, the present investigation was the only controlled, randomized, blocked, blind design using an unplanned population of dairy cattle, in which most were Holstein breed, that showed that ASP during the embryonic period was not associated with atresia coli and/or jejuni. However, from the current research, it is not possible to rule out, as it was suggested initially by Müller et al. [33] that ASP in a genetically susceptible population could cause atresia coli. It would be interesting to use putative carrier females inseminated with semen from putative sires and evaluate the pregnancy status using the present experimental design and follow-up throughout gestation and calving.

In the authors' dairy reproduction practice, pregnancy diagnosis by ASP, in general, is not used. However, calves with atresia coli and/or jejuni were diagnosed from pregnant females that underwent PRP using only the detection of the allantochorion membrane during the embryonic (≤ 45 days) or early fetal period (≥ 46 –90 days), throughout the second trimester of gestation (91–180 days) or only by TRUS at different times of gestation. Consequently, these findings clearly suggest that ASP was not associated with these clinical cases. From 26 animals with atresia coli studied in which information from 11 was available, the PRP was accomplished in 10 females when they were between 55 and 90 days of pregnancy and one cow was not examined at all [37]. In Australia, from 12 Friesian calves with atresia coli, ileum, or jejuni submitted to necropsy, only one had a history of PRP at 12 weeks after breeding [69]. In Iran, from 68 cases of intestinal atresia, none of the dams were diagnosed by PRP during pregnancy [70].

Persuasive proof about the genetic predisposition of atresia coli was placed into evidence when the breeding between eight putative carrier bulls and 56 putative carrier females produced 59 normal and eight atresia coli calves, a finding that was not different from the expected number of 8.1 based on estimation that this abnormality was inherited as an autosomal recessive trait at a single locus with two alleles [68]. In the same dairy herd in a contemporaneous group of random mating, only one calf with atresia coli from 628 births (0.16%) was reported. Interestingly, all the calves with atresia coli from planned and random mating were related [68], and the inbreeding coefficients ranged from 1% to 5.1%. The affected calf from the contemporaneous random mating was the most inbred [68]. From 18 calves reported with atresia coli from 2367 births, all were related and 15 were inbred [67]. Interestingly, the usage of frequency of 16 putative carrier sires increased gradually from 10% to 54% from 1974 through 1977, respectively, with the highest animal incidence of atresia coli 3 years after the greatest usage of the putative carrier sires [67]. Benda et al.

[66] reported that 51 of 53 clinical cases of atresia coli were related to one bull. Eleven surgically corrected female calves with atresia coli produced 32 normal calves and one calf with atresia coli [37]. The transfer of embryos collected from a superovulated putative carrier female inseminated with semen of a putative carrier bull generated three male pregnancies and one that was diagnosed with atresia coli [71]. Moreover, the breeding of five putative carrier cows with three putative bull carriers produced 14 normal offspring and one that presented only tail agenesis [68]. Tail agenesis has been associated with atresia coli in cattle [37,70,72,73]. Willer et al. [74] based in the retrospective analysis of 93 families containing 6416 descendants, reported a heritability coefficient of 0.0875. The minimum gene frequency of the defective allele for atresia coli calculated was 0.025 among registered US Holstein females [75]. Conversely, there is little evidence against the hereditary transmission of atresia coli that was diagnosed in only one of two twin Simmental calves considered identical (based on equal blood type and electrophoretic patterns for hemoglobin, amylase, and transferrin); the other calf was clinically normal [76]. There is also unpublished information about a splitting embryo that produced identical twins, only one of which presented with clinical atresia coli [72]. Therefore, on the basis of all of this information, there is a probable genetic predisposition in certain lines of Holstein. In the present investigation, the two calves born with atresia coli were Holstein and no cases of atresia coli were observed in the non-Holstein breed and crosses. Moreover, the prevalence of atresia coli was reported to be higher in the Holstein breed compared with other dairy or beef breeds [40]. Nevertheless, more studies are warranted to investigate this matter.

In general dairy practices, the veterinarian performs the PRP for early pregnancy diagnosis during the late embryonic period before the potential second estrus (≤ 45 days) [1], for technical and economic reasons [1,8]. Thus, it is very important to have accurate evidence-based medicine about the potential deleterious effect of PRP on pregnancy loss, calving rates, or abnormalities in newborn calves. This information will not only affect the way that the veterinarians practice but also how the owner or manager will perceive the use of this technique for reproductive management. Some authors stated previous recommendations avoiding PRP of the uterus during the first 45 days of gestation [31–33,40,41]. However, the present investigation supported the theory that ASP for pregnancy diagnosis, when performed by a trained veterinarian in a random dairy cattle population, was a safe procedure for the conceptus using the three assessment points: at reexamination, calving, and evaluation of newborn calves.

It was concluded that ASP during the late embryonic period for pregnancy diagnosis in lactating dairy cows did not increase the pregnancy loss, affect calving rates, or produce calves with atresia coli and/or jejuni in dairy cattle.

Acknowledgments

This research was supported by competitive funds from the Department of Large Animal Clinical Sciences (RGS 13-16) of the College of Veterinary Medicine and Biomedical

Sciences of Texas A&M University (Project: Effect of amniotic sac palpation per rectum for pregnancy diagnosis on pregnancy loss and congenital abnormalities in newborn calves). The authors also thank Dr Kathrin Burke for the translation of German articles. They also thank Drs K.G. Solano Suarez and D. Merchan for the invaluable help performing the necropsy in the premature and stillborn calves in farm B. The authors express sincere gratitude to Aurora Organic Dairy to permit and facilitate the present investigation at Cold Water Farm, Stratford, TX, USA. The present investigation was accepted as an abstract for oral communication at the European Buiatric Forum at Rome, Italy on October 14 to 16, 2015.

Competing interests

All authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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