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Comparison between allantochorion membrane and amniotic sac detection by per rectal palpation for pregnancy diagnosis on pregnancy loss, calving rates, and abnormalities in newborn calves



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ABSTRACT

The objectives of the present investigation were to evaluate the pregnancy diagnosis by detection of either the allantochorion membrane (FMS) or amniotic sac (ASP) by per rectum palpation (PRP) during late embryonic or early fetal period on pregnancy loss (PRL) at reexamination, calving rates, and abnormalities in newborn calves. A controlled randomized blind design with 800 lactating dairy pregnant cows diagnosed by transrectal ultrasonography (TRUS) between Days 35 and 57 of gestation from one dairy farm were included. The cows were randomly divided according to detection of allantochorion membrane (FMS group; $n = 264$), detection of amniotic sac (ASP group; $n = 266$), and TRUS (control [CON] group; $n = 270$). TRUS was considered as the criterion standard method of comparison. The entire PRP was performed by one experienced veterinarian. Then, all the cows were reexamined only by TRUS between 2 and 4 weeks later by two independent veterinarians to assess PRL. The calving rate one (number of cows calved divided by the number of cows initially pregnant) and calving rate two (number of cows calved divided by the number of cows pregnant at reexamination) for each group was calculated. All abortions and stillborns were necropsied, and calves alive were followed for 5 days. The overall initial PRL (between initial pregnant cows and reexamination) for FMS, ASP, and CON groups was 7.4% (19/258), 8.8% (23/262), and 9.2% (24/260), respectively ($P = 0.75$). The overall late PRL (between reexamination and calving) for FMS, ASP, and CON groups was 4.2% (9/213), 5.7% (12/209), and 4.2% (9/216), respectively ($P = 0.71$). The calving rate one for FMS, ASP, and TRUS groups was 79.1% (204/258), 75.2% (197/262), and 79.6% (207/260), respectively ($P = 0.63$). The calving rate two for the same groups was 85.4% (204/239), 82.4% (197/239), and 87.7% (207/236), respectively ($P = 0.27$). The number of fetuses aborted late, premature, and mature dead from FMS, ASP, and CON groups was 6, 4, and 5, respectively ($P = 0.85$), and no abnormalities at necropsy were detected. One stillborn male calf with atresia coli after 281 days of gestation from a cow

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examined by ASP at Day 51 was diagnosed. It was concluded that the use of either FMS or ASP for pregnancy diagnosis during late embryonic or early fetal period did not increase the PRL, affect calving rates, or produce calves with congenital abnormalities.

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1. Introduction

The principal objective of a reproductive health program is to ensure that healthy cows calve at 12- to 14-month intervals to optimize their lifetime milk production [1,2]. To accomplish this purpose, cows must become pregnant within 85 to 145 days after calving. Two capital phases in dairy reproductive management are not only breeding cows after the voluntary waiting period but also having an early pregnancy diagnosis [2]. Diagnosis of nonpregnancy before the second probable estrus permits for an earlier management decision [3,4] and reduces the days a cow is not pregnant [5], which leads to shortened and more profitable calving intervals [6].

In current cattle practices, two procedures permit immediate diagnosis of pregnancy: per rectum palpation (PRP) and transrectal ultrasonography (TRUS) [2–4]. Pregnancy diagnosis by PRP is the most frequent procedure used by veterinarians around the world [3,7–9], although other techniques of pregnancy diagnosis are available [2,10–13]. In USA, according to the last report of National Animal Health Monitoring System [9], 93% of the Dairy Operations performed pregnancy diagnosis and 86% of these operations used PRP to perform pregnancy diagnosis. Almost 90% of the operations used a veterinarian to perform these pregnancy exams. No differences among small (<100 heads), medium (101–499), and large (>500) dairy operations in the use of PRP were detected. More than one-fourth of operations (27.4%) routinely used TRUS with PRP to assess pregnancy status. Moreover, PRP was used to diagnose pregnancy on 96.3% of operations in the west region, compared with 84.8% in the east region of the country. Per rectum palpation continues to be the most used technique for pregnancy diagnosis for practitioners. The use of PRP is based on multiple reasons such as not requiring equipment or laboratory and the fact that the results are fast, permitting an immediate decision. It is an accurate technique after Day 35 of breeding when performed by trained veterinarians; it permits aging the pregnancy and can assess the viability of the fetus. In addition, it is cheaper when compared with other techniques, gives additional information about other internal organs, and permits evaluation of body condition score, cleaning score, leg conformation, and udder [3,4,8,14,15]. Per rectum palpation for pregnancy diagnosis is based on the detection of at least one of the four positive signs of pregnancy such as allantochorion, amniotic vesicle, placentomes, and/or fetus [3]. During earlier stages of gestation detection, either the amniotic sac or allantochorion membrane (also known as fetal membrane slip technique; FMS) is used as a positive sign of pregnancy [16,17]. The size of the amniotic sac in relationship to the fingers or size of hand of the veterinarian permits one to estimate the age of pregnancy during the first 65 to 70 days of pregnancy [18]. The diagnosis of twin pregnancies requires the assessment

of the number of amniotic sacs [3,8,19]. Interestingly, until recently, few studies were designed to answer two essential aspects of PRP such as safety and accuracy [4,10,15,20–22].

In regards to the safety aspects for the conceptus by PRP, contradictory proofs were published [4,21]. Investigators in some studies suggested that PRP had little or no effect on pregnancy loss (PRL) [23–25]. Conversely, investigators in other reports suggested that PRP during early gestation increased PRL [26–31]. However, all these studies had important limitations in their design as previously reported [4]. In recent controlled studies, it was shown that the detection of either the allantochorion membrane or amniotic sac by PRP through the embryonic period did not increase the PRL when reexamined by TRUS in the course of the fetal period [4,21,22]. However, in those trials, no information about calving rates or the clinical status of the newborn calves was reported. In a number of investigations, initially from the United States, then from Germany and later on other locations, an association between amniotic sac palpation (ASP) during the embryonic period for pregnancy diagnosis (till Day 45 of gestation) [32], especially between 36 and 42 days, an increased risk of atresia coli/jejuni in newborn calves [33–38] was observed. In atresia coli/jejuni, a section of the large bowel or jejunum is absent, resulting in a blind ending intestinal tube. This clinical congenital condition is lethal, and surgical correction is the only treatment available [39–42]. Atresia coli/jejuni has been reported in different countries and reported in more than 10 breeds of cattle, with a marked preponderance in Holstein calves [43]. Based on those findings, some sources have recommended avoiding PRP of the uterus during the first 45 days of gestation [33–38]. In spite of this, intestinal atresia was also reported to be inherited as an autosomal recessive trait in Jersey and Swedish Highland cattle [44,45]. Intestinal atresia could develop either from imperfect canalization of the gut or from insufficient blood supply to the affected portion of the intestine [46]. This last mechanism was suggested to be caused by ASP [36,43]. Nonetheless, the root of atresia coli/jejuni remains polemic and not completely understood [36,43,46]. In a recent controlled randomized blind experiment performed in two dairy operations with 680 pregnant cows to evaluate the effect of ASP during the embryonic period, no differences on PRL, calving rates, and abnormalities of newborns were detected [47]. Remarkably, two calves were born with atresia coli only in the CON group (which only received TRUS throughout gestation). This is strong evidence against the harmful effect of ASP for pregnancy diagnosis on abnormalities in newborn calves. Also, in the author's practice, the use of ASP for early pregnancy diagnosis is not routinely used. Conversely, calves with atresia coli/jejuni were diagnosed among newborn calves from females that underwent PRP only by the detection of allantochorion membrane, either during the embryonic period or fetal period, females diagnosed as

pregnant using only TRUS as well as females that never were submitted to PRP throughout gestation (Romano, unpublished data). These observational findings powerfully suggest that ASP during early gestation was not associated with this pathological condition. However, to the best of the authors' knowledge, no controlled studies were available with the initial pregnancy diagnosis by ASP performed during the early fetal period (≥ 46 –60 days) on PRL, calving rates, and abnormalities in newborn calves. Moreover, no evidence was published about the use of allantochorion membrane detection on calving rates or abnormalities in newborn calves. TRUS permits an immediate, early, and accurate method for early pregnancy diagnosis and has been reported as not affecting embryo or fetus viability or producing abnormalities in the newborn [2,48–52]. Therefore, with the assistance of TRUS, one could potentially reduce or eliminate the PRP of the uterus, permitting a better experimental design to create a CON group of contemporaneous pregnant females to study the effect of allantochorion membrane or amniotic sac detection by PRP to investigate these two techniques on PRL, calving rates, and abnormalities in newborn calves.

The objectives of the present study were to evaluate the effect of PRP for pregnancy diagnosis by detection of either the allantochorion membrane or the amniotic sac during late embryonic or fetal periods on PRL at reexamination, calving rates, and abnormalities in newborn calves.

2. Material and methods

2.1. Study population/animals

The present investigation was performed in one dairy operation located in the Texas Panhandle during March 2014 to January 2015. Eight hundred lactating pregnant cows from the Cold Water Dairy Farm from Strafford, TX, USA, were included. The study was performed in compliance with established standard operating procedures and guidelines for animal care and use of Texas A&M University. The average number of days between calving and fertile breeding dates was calculated for each group of cows. The average lactation number was 2.4 ± 1.3 (range 1–9). Body condition scores were assessed at the time of initial pregnancy diagnosis, and the scale used was from 1 to 5 [53].

Each lactating cow was artificially inseminated after a voluntary waiting period of 45 days. No estrous synchronization protocol was used. Cows were subject to a reproductive program based on insemination from visual estrus detection. Cows' tail heads were painted daily with color chalk and checked for estrus by removal of tail chalk. If estrus was determined, cows were artificially inseminated during the morning. The females were inseminated with frozen-thawed semen from different Holstein bulls. The females were housed in a free-stall system. The vaccination protocol was scheduled at the following periods: 25 to 30 days postpartum with *Escherichia coli* (*E coli*) bacterin (*Enviracor J-5*; *E coli* Bacterin; Zoetis Animal Health, Kalamazoo, MI, USA), infectious bovine rhinotracheitis, bovine viral diarrhea types 1 and 2, parainfluenza 3, bovine respiratory syncytial virus, *Campylobacter fetus* and *Leptospira canicola*, *grippotyphosa*, *hardjo*,

icterohaemorrhagiae, *pomona* and *borgpetersenii* (*Bovishield Gold 5 FP L5 HB*; Zoetis Animal Health). At pregnancy diagnosis, cows were vaccinated with *Lepstopira bacterin* containing *Leptospira canicola*, *grippotyphosa*, *hardjo*, *icterohaemorrhagiae*, *pomona* (*Leptoform 5*; Zoetis Animal Health); at dry-off (–60 days), with *Clostridium chauvoei*, *septicum*, *haemolyticum*, *novyi*, *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). Finally, at parturition (–15 days), another booster of *E coli* bacterin was administered (*Enviracor J-5*; *E coli* Bacterin; Zoetis Animal Health).

Lactating cows were milked three times a day. Diets were formulated to meet or exceed National Research Council requirements [54]. Water was provided ad libitum. Pregnant cows that developed clinical or subclinical mastitis, lameness (≥ 3 on a scale of 1–5) [55], or digestive disorders (e.g., diarrhea) from the day of pregnancy diagnosis to the end of the study were treated accordingly.

2.2. Experimental design and research methods

The present experiment was a randomized controlled double-blind design. Cows were diagnosed as pregnant based on the presence of a viable embryo by TRUS between Days 35 to 60 after breeding and [2] then were divided randomly into three groups: CON group, allantochorion membrane detection by PRP (FMS group), and amniotic sac palpation PRP (ASP group). All the initial TRUS examinations were performed by the same veterinarian in the morning, who used a portable ultrasound machine equipped with a 7.5-MHz linear transducer as previously described [21]. The CON group did not receive any PRP of the uterus. The FMS group was submitted to PRP to detect the allantochorion membrane by compression of the pregnant uterine horn and allowing the chorioallantoic membrane to slip between the fingers [4]. 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 [15]. All the initial pregnancy diagnoses were performed only once by a board-certified theriogenologist with over 30 years of bovine practice. In the event that one amniotic sac was found, no attempt was made to look for a second amniotic vesicle; therefore, the possibility of twin pregnancy detection was avoided. In general, the uterine horns were retracted directly or indirectly before this approach was used. No person was allowed to perform PRP or TRUS on the cattle at any time during the experimental period. After submitted to their respective treatments, each female was reevaluated for pregnancy again, in general, 2 to 4 weeks later. These pregnancy reexaminations were performed only by TRUS by two different veterinarians from the initial pregnancy diagnosis who were blind to the treatment of each cow but were aware of this project. All the pregnant females at this stage were followed until calving. A diagnosis of PRL was made when a heartbeat or

signs of pregnancy (allantochorion membrane, amniotic sac, conceptus, or placentomes) were not seen by ultrasonography, or signs of conceptus degeneration were observed by TRUS [56]. All abortions, premature, or mature dead calves were submitted to necropsy to determine if abnormalities were present and which type. The necropsies were performed by two veterinarians, different from those of initial examination or reexaminations, who were also blinded to the initial intervention. All calves alive at birth were maintained for observation for 3 to 5 days to detect any type of abnormalities.

Initial PRL was defined as the PRL between initial PRP and reexamination that include both late embryonic and early fetal period. Late embryonic PRL rate was defined at the percentage between the number of cows with PRL at reexamination and the initial number of pregnant cows assessed by PRP during late embryonic period. Early fetal PRL was defined as the percentage of PRL between the number of cows with PRL at reexamination from the initial number of pregnant cows assessed by PRP during early fetal period. Late PRL rate was the percentage of PRL between the number of cows that did not calve and the number of cows that were pregnant at reexamination. This is a measure of prenatal fetal loss from the middle of the first trimester of gestation to calving. Calving rate one was the percentage between the number of females that calved and the initial number of pregnant females. Calving rate two was the percentage between the number of cows that calved and the number of pregnant cows at reexamination. Missing cow was considered when the cow in the follow-up was either not found at the correct time, or sold or died.

2.3. Statistical analysis

The null hypothesis was that PRP for pregnancy diagnosis either by detection of amniotic sac or allantochorion membrane during embryonic or fetal period was no different from TRUS on proportion of PRL at reexamination, calving rates, and abnormalities in the newborn calves. The minimum sample size required for a two-sided alternative hypothesis to detect a difference in PRL of 10% among groups with a PRL of 10% between initial pregnancy diagnosis and reexamination by use of α error = 0.05 and β error = 0.10 (power = 90%) was 265 cows in each group [57,58]. In the design phase of this investigation, a power of 90% was chosen to decrease the probability of discovering “no difference” when real differences could exist (reduce potential false negative results); the main goal was to support the null hypothesis = no difference among treatments when the alternative hypothesis was tested. In multiple independent studies [4,13,21,47], the designs were constructed to detect differences in PRL between 5% and 10% between groups using an error = 0.05 and a β error between 0.2 and 0.1 (power 80% or 90%, respectively), and no divergences between PRP and control groups were detected. The proportion of PRL and calving rates for cows subjected to ASP, FMS, or TRUS was compared with the proportion for CON during the two periods by use of chi-squared analysis or Fisher’s exact test as appropriate [57,59]. The continuous variables (body condition score, lactation number, and postpartum days at artificial

insemination) were analyzed by one way of analysis of variance [58,59]. Every cow that was missing was not included in the statistical analysis. All data were expressed as mean \pm 1 standard deviation (range). Values of $P \leq 0.05$ were considered significant. A software program was used to analyze all data sets [57].

3. Results

The average \pm standard deviation (range) lactation number for FMS, ASP, and CON groups was 2.4 ± 1.3 (1–8), 2.5 ± 1.5 (1–9), and 2.4 ± 1.3 (1–7), respectively ($P = 0.44$). The average body condition score at the initial pregnancy diagnosis for the same groups was 2.5 ± 0.3 , 2.5 ± 0.3 , and 2.6 ± 0.3 , respectively ($P = 0.51$). The average days from postpartum to artificial insemination for FMS, ASP, and CON groups were 105.2 ± 61.3 , 101.7 ± 49.0 , and 105.7 ± 55.4 , respectively ($P = 0.67$). The average days at initial pregnancy diagnosis for FMS, ASP, and CON groups were 43.8 ± 5.6 (35–57), 44.4 ± 5.9 (35–57), and 44.0 ± 5.9 (35–57), respectively ($P = 0.41$). The average days for TRUS reexamination for the same groups were 59.1 ± 7.8 (49–109), 59.1 ± 7.8 (49–79), and 59.6 ± 8.6 (49–117), respectively ($P = 0.76$). The number of missing animals between initial pregnancy diagnosis and reexamination from groups FMS, ASP, and CON was 6, 4, and 10, respectively ($P = 0.25$). Late embryonic PRL (Days 35–45) for FMS, ASP, and CON groups was 7.7%, 8.9%, and 12.4%, respectively ($P = 0.33$; Table 1). Early fetal PRL (Days 46–57) for FMS, ASP, and CON groups was 6.8%, 5.0%, and 5.4%, respectively ($P = 0.80$). The overall initial PRL (late embryonic plus early fetal period examinations; Days 35–57) for FMS, ASP, and CON groups was 7.4% (19/258), 8.8% (23/262), and 9.2% (24/260), respectively ($P = 0.75$). Differences were detected ($P = 0.04$) in PRL between all the groups that were evaluated at the late embryonic period (10.1%; 49/486) and for those evaluated at the early fetal period (5.8%; 17/294). The number of missing animals from reexamination to calving from groups FMS, ASP, and CON was 26, 30, and 20, respectively ($P = 0.43$). Late PRL between the cows pregnant at reexamination from the late embryonic period evaluation (Days 35–45) and calving for FMS, ASP, and CON groups was 3.2%, 6.4%, and 3.0% respectively ($P = 0.32$; Table 2). Late PRL between the cows pregnant at reexamination from the early fetal period evaluation (Days 46–57) and calving for FMS, ASP, and CON groups was 6.0%, 5.1%, and 6.6%, respectively ($P = 0.94$). The late PRL rate from all groups between cows pregnant at reexamination and calving from all the groups that were from the embryonic period (4.1%) to the early fetal period (5.5%) was not different ($P = 0.41$). The overall late PRL rate between reexamination (from the embryonic period plus early fetal evaluation; Days 35–57) and calving for FMS, ASP, and CON groups was 4.2% (9/213), 5.7% (12/209), and 4.2% (9/216), respectively ($P = 0.71$). Initial PRL was higher (8.5%; 66/780) than late PRL (4.7%; 30/638; $P = 0.005$). The calving rate one was 77.3% for the FMS group, 74.1% for the ASP group, and 76.9% for the CON group ($P = 0.63$; Table 3). The calving rate two for the same groups was 85.4%, 82.4%, and 87.7%, respectively ($P = 0.27$). The number of fetuses aborted, premature and mature dead from FMS, ASP, and

Table 1

Early pregnancy loss from allantochorion membrane detection (FMS), amniotic sac detection (ASP), and control group (CON) from lactating dairy cows examined by per rectum palpation during late embryonic period or early fetal period.

Group	FMS	ASP	CON	All
Initial number of pregnant cows	264	266	270	800
Missing females from initial diagnosis to reexamination	6	4	10	20
Number of cows with pregnancy loss at reexamination evaluated at late embryonic period (%)	12 (7.7) ^a	18 (11.1) ^a	19 (11.3) ^a	49 (10.1) ^b
Number of cows with pregnancy loss at reexamination evaluated at early fetal period (%)	7 (6.9) ^a	5 (5.0) ^a	5 (5.4) ^a	17 (5.8) ^c
Number of cows with pregnancy loss from initial examination to reexamination (%)	19 (7.4) ^a	23 (8.8) ^a	24 (9.2) ^a	66 (8.5)
Number of cows pregnant at reexamination from the evaluation at late embryonic period	144	144	149	437
Number of cows pregnant at reexamination from the evaluation at early fetal period	95	95	87	277
Total number of females pregnant at reexamination	239	239	236	714

^aRows with the same superscript letter are not significantly different ($P \geq 0.05$).

^{b,c}Columns with different superscript letters are significantly different ($P = 0.04$).

CON groups was 6, 4, and 5, respectively ($P = 0.85$); no abnormalities were detected at the necropsy of these animals. One male calf was stillborn with atresia coli confirmed at necropsy (see Fig. 1; $P = 0.49$). This calf was born at Day 281 of gestation and both parents were Holstein. The dam was from the ASP group and examined at Day 51 of gestation (estrus = day 0).

4. Discussion

The present investigation compared, for the first time under a controlled randomized double-blind design in one dairy operation, two techniques for pregnancy diagnosis, either FMS or ASP, during late embryonic or early fetal period contrasted concurrently with a CON group of pregnant cows of the same gestational age. PRLs at reexamination between FMS and ASP groups were not different from the CON group. These outcomes were in agreement with earlier controlled studies. However, in those previous reports, either FMS was compared with TRUS [4,21] or ASP was contrasted with TRUS [15] but, not all these procedures were judged simultaneously. In former, noncontrolled studies, when FMS was compared with ASP between Days 30 and 44 after breeding, a PRL at recheck of 4.8% and 5.1% was reported [60], and similar results were stated by Studer [61]. In a follow-up study, when ASP was performed between Days 35 and 50 and the females

were reexamined at 5 m later, a PRL of 3.6% [62] and 10% [63] was noticed. In a later study, a PRL of 19% between pregnancy diagnoses by ASP performed between Days 30 and 45 and calving was described [30]. In regard to FMS, in multiple noncontrolled studies, the procedure was shown to be deleterious for the conceptus [26–28]. However, the major limitation in all those earlier studies that reported an association between PRP and PRL was that the source of information was based on observational findings; none of these studies included a CON group of contemporaneous non-PRP group at the same gestational period for comparison. Consequently, it was not possible to differentiate between the spontaneous PRL and the potential effects of the technique of pregnancy diagnosis.

No difference among techniques of pregnancy diagnosis on prenatal PRL was detected. In a former investigation that compared three techniques of pregnancy diagnosis, fluctuation, allantochorion detection, and amniotic sac detection by PRP, it was concluded that allantochorion membrane detection increased significantly the PRL compared with the other two procedures [26]. It is difficult to interpret how FMS produced the highest PRL compared with ASP, which required more manipulation of the uterus and skill to detect the amniotic sac. The allantochorion membrane is an external sac in juxtaposition with the endometrium and, after 40 days, is detectable in both

Table 2

Late pregnancy loss from allantochorion membrane detection (FMS), amniotic sac detection (ASP), and control group (CON) from lactating dairy cows examined by per rectum palpation during late embryonic period or early fetal period.

Group	FMS	ASP	CON	All
Number of pregnant females at reexamination	239	239	236	714
Number of pregnant females at reexamination from embryonic period	144	144	149	437
Number of pregnant females at reexamination from early fetal period	95	95	87	277
Missing females from reexamination to calving	26	30	20	76
Total number of cows with pregnancy loss between reexamination and calving	9 (4.2) ^a	12 (5.7) ^a	9 (4.2) ^a	30 (4.7)
Number of cows with pregnancy loss between reexamination and calving evaluated at late embryonic period	4 (3.2) ^a	8 (6.4) ^a	4 (3.0) ^a	16 (4.2) ^b
Number of cows with pregnancy loss between reexamination and calving evaluated at early fetal period	5 (5.7) ^a	4 (4.8) ^a	5 (6.2) ^a	14 (5.6) ^b
Animals that calved from evaluation at late embryonic period	121	118	131	370
Animals that calved from evaluation at early fetal period	83	79	76	238
Total number of cows that calved	204	197	207	608

^aRows with the same superscript letter are not significantly different ($P \geq 0.05$).

^bColumn with the same superscript letter is not significantly different ($P \geq 0.05$).

Table 3

Calving rates one^a and two^b from allantochorion membrane detection (FMS), amniotic sac detection (ASP), and control group (CON) from lactating dairy cows examined by per rectum palpation during late embryonic period or early fetal period.

Group	Initial number of pregnant cows	Total number of females pregnant at reexamination	Total number of cows that calved	Calving rate one (%) ^a	Calving rate two (%) ^b
FMS	264	239	204	77.3 ^c	85.4 ^c
ASP	266	239	197	74.1 ^c	82.4 ^c
CON	270	236	207	76.9 ^c	87.7 ^c
All	800	714	608	76.0	85.2

^cColumns with the same superscript letter are not significantly different ($P \geq 0.05$).

^a The percentage between the number of cows that calved and the initial number of cows pregnant.

^b The percentage between the number of cows that calved and the number of cows pregnant at reexamination.

uterine horns by PRP. On the other hand, the amniotic sac, in a singleton, occupies a small part of the pregnant uterine horn that requires thorough examination for its detection, demanding simultaneously gentle and indirect pressure to avoid producing iatrogenic abortion [3,15,61].

The percentage of PRL was higher in the late embryonic period compared with the early fetal period and in concordance with preceding investigations [4,15,21]. These two periods had higher PRL compared with later periods. This information is critical because the earlier that a female is diagnosed as pregnant, the greater the chance that a pregnant female will have spontaneous PRL unrelated to the technique used for pregnancy diagnosis. Consequently, the detection of a pregnant female during early stages of pregnancy, especially during the late embryonic period or early fetal period, will require obligatory reexamination to

avoid maintaining, later on, a nonpregnant female in the production system. In addition, the PRL during early gestation period, in general, will be most likely unnoticed [63,64]. Finally, grounded from the present findings as well as other studies [56,65], diagnosis of a female as pregnant at reexamination does not guarantee that she will calve; therefore, an additional reexamination needs to be included. Pregnancy diagnosis is a dynamic clinical condition; a female could maintain the pregnancy or lose it. On the other hand, nonpregnancy is a static condition that needs assessment to place the female as soon as possible in the reproductive program [21]. PRL varied among farms [21,28,56,61,64]; therefore, different policies of pregnancy diagnosis and reexamination need to be established for each dairy operation.

The pregnancy diagnosis by detection either of amniotic sac or allantochorion membrane was executed as typically performed under private practice. That means that only one veterinarian performs one examination looking for only one positive sign of pregnancy. This fact could be judged as a limited factor; however, this person has the experience and credentials to be considered as a regular bovine practitioner. On the other hand, the effect of variation among clinicians on PRL as was previously reported was reduced or eliminated by using one trained veterinarian [26]. In previous studies, it seems that a more aggressive approach was used, because pregnant females underwent PRP from more than one person. In addition, multiple techniques were used in the same animal at the same examination, or several techniques were used by more than one person in the same pregnant female [26–29]. Most of these studies were derived from clinical educating programs that included less experienced people or more rough and extensive assessment of the female genital tract was performed.

In earlier controlled investigations, the calving rates were not evaluated [4,15,21]. Meanwhile, in all the studies reported, there was not a CON group for comparison [27,30,62,66]. The present outcomes are in concordance with a recent investigation in which ASP was used only during the late embryonic period and compared with a concurrent CON group of pregnant females throughout the gestation with no differences in calving rates detected [47]. Therefore, the present experiment not only agreed with this last study but also extends knowledge that the use of FMS for pregnancy diagnosis affected neither the late PRL nor calving rates.

Based on the current outcomes, it was proved once more, the importance of the experimental design

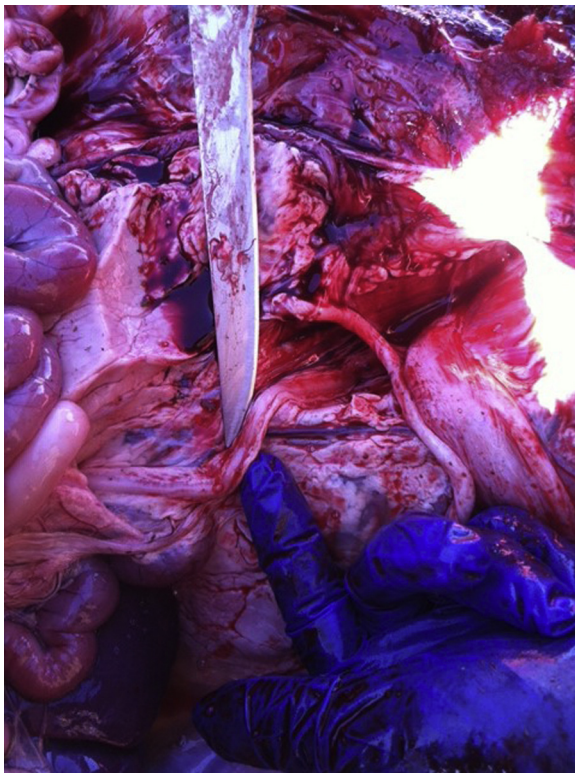


Fig. 1. The necropsy is showing the area of atresia coli that is between the tip of the knife and the forefinger.

containing an adequate concurrent CON group of comparison to arrive at sound conclusions [4,15,21]. The presence of a contemporaneous cluster of pregnant females of the same age of gestation that did not undergo PRP for comparison permitted differentiation between the spontaneous PRL in the CON group from the potential effects of the technique by PRP for pregnancy diagnosis groups. This experimental approach was only possible because of the availability of TRUS, which permitted an immediate, early, and accurate method of pregnancy diagnosis [2]. Moreover, there is no report that TRUS showed any detrimental effect on the conceptus under normal conditions of use [48–52].

In preceding noncontrolled studies of dairy cows assessed by ASP during late embryonic period, the prevalence of atresia coli reported was 5.3% [35], 4.8% [36], 5.1% [67], 2.5% [68], and 6.9% [38]. Therefore, based on all these reports, it was expected that when ASP was performed during the high-risk period of pregnancy, between Days 35 and 45, the frequency of atresia coli will be significantly higher compared with FMS or CON groups. It was expected that between four and 11 calves would be diagnosed with atresia coli. However, no calves with atresia coli were detected in 158 cows evaluated. This outcome is in agreement with the findings of a recent study in which ASP was performed in 341 pregnant cows during the late embryonic period (≤ 45 days); of these, 285 were between Days 35 and 42. No cases of atresia coli were identified [47]. Only two calves with atresia coli were diagnosed in the CON group that involved 337 pregnant cows assessed only by TRUS. The prevalence of this last study was 0.59% for the CON group, and prevalence including all the experimental animals was 0.29% [47]. Therefore, supported by these two studies, there is strong evidence against the deleterious association of ASP during the late embryonic period and atresia coli. Finally, from 26 calves with atresia coli studied, for which history from 11 was available, PRP was performed in 10 females when they were between 55 and 90 days of pregnancy; one cow was not examined at all [40]. In Australia, from 12 calves submitted to necropsy, only one had a record of PRP at 12 weeks after breeding [69]. In Iran, from 68 clinical cases of intestinal atresia, none of the dams were examined via PRP throughout gestation [70].

In the present study, only one calf was diagnosed with atresia coli, and the dam was in the ASP group that was examined during the early fetal period; this was in contrast with all these previous studies that associated ASP during the late embryonic period with atresia coli or atresia jejuni [33,35–38]. In those noncontrolled reports, no calves with atresia coli were reported from 995 cows examined by ASP after Day 40 [35], from 103 females examined after Day 43 [36], from 45 pregnant cows examined between Days 39 and 51 [35], or from 800 pregnant females assessed after Day 43 [38]. In the present study, the calf was born dead from a Holstein dam examined at Day 51 of pregnancy that was artificially inseminated by a Holstein bull. The prevalence of atresia coli for the ASP was 0.38% (1/266), and the overall prevalence for all the groups was 0.13% (1/800). This prevalence is very low. The present outcomes were in conformity with other reports that pregnancy diagnosis performed after Day 40 corresponded to 359 normal calves and one affected calf (0.3%) or a mean prevalence of 0.76%

from a Holstein herd that reported 18 cases of atresia coli from 2367 births (from 1974–1983; range between years from 0%–1.55%/y) [67]. In a different study, from the same herd, one calf with atresia coli (0.34%) was detected from 295 normal calves diagnosed from a contemporaneous random mating group [68]. It is necessary to remark that the major limitation in all these previous reports was that the source of information was based on observational findings because none of these studies included a comparison CON group of contemporaneous pregnant females of the same gestational age that did not undergo PRP. Therefore, PRP was a potential confounding factor rather a causative effect because all females underwent PRP.

In general, the veterinarian in dairy practice performs an early pregnancy diagnosis by PRP using either ASP or FMS during the late embryonic period before the potential second estrus (≤ 45 days) [3,7,8] for technical and economic reasons [3,4,6,61]. There is a need for accurate information supported in evidence-based medicine about the potential harmful effect of PRP on PRL, calving rates, or abnormalities in newborn calves. This knowledge will not only affect the way that the veterinarians practice but also how the owner or manager will perceive the use of this method in his or her reproductive management [4,47]. Some authors stated previous recommendations avoiding PRP of the uterus to detect the amniotic sac during the first 45 days of gestation [33,35–38,71,72]. The present findings support the use of either ASP or FMS for pregnancy diagnosis, when performed by a trained veterinarian in a random dairy cattle population, was a safe procedure for the conceptus using three assessment points: at reexamination, at calving, and at evaluation of newborn calves.

It can be concluded that using either FMS or ASP for pregnancy diagnosis performed during late embryonic or early fetal period, did not increase the PRL, affect the calving rates, or produce calves with congenital abnormalities.

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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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