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journal homepage: www.theriojournal.com



# 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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#### ARTICLE INFO

### Article history:

Received 16 August 2016 Received in revised form 4 November 2016 Accepted 7 November 2016 Available online 12 November 2016

Keywords:
Cattle
Pregnancy
Amniotic sac
Allantochorion membrane
Pregnancy loss
Atresia coli

#### 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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The present investigation was presented to the World Buiatrics Conference at Dublin, Ireland, on July 5th, 2016.

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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 jejuni 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]. Inspite 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

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