Early pregnancy diagnosis by palpation per rectum:
Influence on embryo/fetal viability in dairy cattle

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Abstract

The objective was to estimate the effect of palpation per rectum (for early pregnancy diagnosis) on embryo/fetal viability in dairy cattle. A controlled, randomized block-design experiment with two blocks, one by category, and the other by number of embryos, was conducted. Five-hundred-and-twenty pregnant dairy cows and heifers with a viable embryo detected by transrectal ultrasonography (TRUS) between days 29 and 32 after AI were included. The pregnant females were randomly allocated into two nearly equal groups: palpation per rectum (PAL group; n = 258) and no palpation per rectum (NPAL group; n = 262). The PAL group was submitted to palpation per rectum (PPR) using the fetal membrane slip (FMS) technique once between days 34 and 41 of pregnancy. The fetal membrane slip consisted of compressing the pregnant uterine horn and allowing the chorioallantoic membrane to slip between the fingers. Both groups were submitted to two additional TRUS at days 45 and 60 of pregnancy, to monitor the potential immediate and delayed deleterious effects of PPR on embryo and fetal viability, respectively. A diagnosis of embryo/fetal death was made when there was no embryo/fetal heart beat or the absence of positive signs of pregnancy in an animal previously diagnosed pregnant, or the presence of signs of embryo/fetal degeneration. The overall rate of embryo/fetal death was 14.0% (73/520). Embryonic death (10%; 52/520) was higher than fetal death (4.5%; 21/468; P < 0.001). Embryo/fetal mortality was higher in cows (16.4%; 59/360) than in heifers (8.8%; 14/160; P < 0.025) and in cattle with twin (25.5%; 12/47) versus singleton pregnancies (12.9%; 61/473; P < 0.025), but was not different (P > 0.05) between PAL (14.7%; 38/258) and NPAL (13.4%; 35/262). In conclusion, PPR between days 34 and 41 of pregnancy using the fetal membrane slip technique did not affect embryo/fetal viability.

Keywords: Dairy cattle; Pregnancy diagnosis; Palpation per rectum; Fetal membrane slip; Embryonic loss

1. Introduction

In bovine practice, palpation per rectum (PPR) is one of the most frequent procedures performed by veterinarians and is the most frequent method used for pregnancy diagnosis [1–3]. A skilled practitioner is able to detect pregnancy in cattle as early as day 35 [1–6].
The importance of a systematic and non-traumatic technique of palpation per rectum cannot be over-emphasized; it is well known that embryonic/fetal deaths can be induced accidentally or iatrogenically by this procedure [7–10]. Early pregnancy diagnosis is vital for efficient reproductive cattle management [4]; ideally, it should correctly identify both pregnant and non-pregnant females.

There is contradictory information regarding the potential deleterious effects of palpation per rectum for early pregnancy diagnosis on embryo/fetal viability. Early pregnancy diagnosis is considered when the diagnosis is performed 45 days after breeding [4]. Some studies suggested a possible adverse effect of early palpation per rectum [11–16]. In contrast, other studies [17,18] suggested that the time at which the first palpation per rectum was performed after insemination had little effect on the calving rate. These studies had several weaknesses in their design. Previous reports that diagnosed pregnant females by palpation per rectum [11–13], progesterone [14], or protein B [19,20] did not assess the viability of the embryo/fetus. Most of the studies lack a “pregnant non-palpated control group” [11–13] to differentiate the effects of palpation per rectum from spontaneous embryo/fetal death occurring during early pregnancy [21]. The interval between palpation per rectum and re-evaluation was variable: from 30 to 90 days [11], 44 to 48 days [14], or at calving [12], or variable depending if the palpation was performed before or after day 40 [13]. This is important because the embryo/fetal can be affected by factors other than palpation per rectum. With regard to the use of progesterone for early pregnancy diagnosis, progesterone concentrations are high in conditions other than pregnancy, such as the presence of luteal cysts, long estrous cycles, sampling during the luteal phase, and pyometra [22], as well as in pregnant females with embryo/fetal death [23]. In addition, progesterone concentration is a better indicator of “non-pregnancy” status than of pregnancy status [24,25]. Bovine pregnancy specific protein B (bPSPB), a glycoprotein produced by the trophoblast, remains elevated despite embryo/fetal death or embryos that are in the process of degeneration [26,27]. For induced embryo/fetal mortality, elevated concentrations of progesterone as well as positive signs of pregnancy persisted for several days or weeks, despite the occurrence of embryo/fetal death [10,23]. In a recent study, persistence of an elevated protein B concentration was detected in spontaneous embryo/fetal mortality in dairy cattle [28]. Although differences in the incidence of pregnancy loss among farms are well established and are more related to management factors than to infectious diseases [29], this was not taken into consideration in some studies. Most of the previous reports combined heifers with cows. One study showed that pregnant heifers have lower embryo/fetal mortality rates than cows [30]. Previous studies did not report the number of twin pregnancies. Twin pregnancies increase the risk of embryo/fetal death and abortion [31]. Moreover, in previous reports, practical conditions of palpation per rectum were not followed. In routine clinical practice, each female is palpated per rectum once by only one person looking for positive signs of pregnancy. However, in these previous reports the females were palpated per rectum consecutively by more than one person at the same time, different techniques were used at the same time, or different techniques were used in the same animal by more than one person [11–14]. In the above cases, the procedure of palpation per rectum was more prolonged than that typically used for diagnosis of pregnancy in practice, i.e. each female is palpated per rectum once by a single veterinarian. Finally, sick pregnant females have an increase risk of abortion compared to healthy animals. Cows with clinical mastitis during the first 45 days of pregnancy were 2.7 times more likely to undergo abortion in the following 90 days than cows without clinical mastitis [32].

Confirmation of pregnancy status before or at the time of palpation per rectum by another method allows the differentiation of spontaneously occurring embryonic/fetal loss from embryonic/fetal loss induced by palpation per rectum. The use of transrectal ultrasonography permits an earlier pregnancy diagnosis than palpation per rectum, gives immediate information to confirm pregnancy as well as assess embryo/fetal viability, and reduces the number of false positive diagnoses and false negatives when palpation per rectum is used [33]. In addition, reports about the use of transrectal ultrasonography have shown that it is a safe technique that does not affect the embryo or fetus viability [34–36]. The objective of the present study was to estimate the effect of palpation per rectum using the fetal membrane slip technique for early pregnancy diagnosis on embryo/fetal viability in dairy cattle. The null hypothesis was that palpation per rectum, using the fetal membrane slip technique for pregnancy diagnosis, is not deleterious for the conceptus.

2. Materials and methods

The study was conducted in the Dairy Cattle Center of Texas A&M University from December 1999 to
through March 2002. Holstein and Jersey cows and heifers were used. Each cow was inseminated after the voluntary waiting period of 55 days postpartum. Each heifer was inseminated between 14 and 16 months of age. The breeding program consisted of the administration of prostaglandin F2α (Lutalyse, 25 mg im; Pharmacia & Upjohn Co., New York, NY, USA) every Monday morning after palpation per rectum. Detection of estrus was performed twice a day: early in the morning (between 06:00 and 09:00) and late in the afternoon (between 18:00 and 21:00). Artificial insemination was performed 8–12 h after detection of the first signs of estrus, using Certified Semen Services frozen semen (estrus = day 0). Lactating cows were housed in a covered freestall barn, milked twice daily, and fed a total mixed diet consisting of corn or grain sorghum, soybean meal, alfalfa, and corn silage. Mineral salt and water were provided ad libitum. Diets were formulated to meet or exceed National Research Council requirements (2001; [37]). Body condition scores were assessed during all the periods of investigation, and the scale used was from 1 to 5 [38].

Pregnant cows/heifers that developed clinical mastitis, lameness (≥3 in a scale of 1–5) [39] or digestive disorders (diarrhea, etc.) from estrus synchronization to 60 days of pregnancy were treated but excluded from the study. The vaccination program included only products that contained killed bacteria and viruses. The time of vaccination for cows was scheduled at the following periods: 25–30 days postpartum with Viral Diarrhea types 1 and 2, Parainfluenza 3, Bovine Respiratory Syncytial Virus, Infectious Bovine Rhinotracheitis, Bovine Escherichia coli bacterin-toxoid and Salmonella typhimurium bacterin-toxoid plus immune stimulator (Endovac-Bovi with immuneplus; Immvac, Columbia, MO, USA) and prepartum (−15 days) with Salmonella typhimurium bacterin-toxoid plus immune stimulator (Endovac-Bovi with immuneplus; Immvac, Columbia, MO, USA) and prepartum (−15 days) with Salmonella typhimurium bacterin-toxoid plus immune stimulator (Endovac-Bovi with immuneplus; Immvac, Columbia, MO, USA) and prepartum (−15 days) with Salmonella typhimurium bacterin-toxoid plus immune stimulator (Endovac-Bovi with immuneplus; Immvac, Columbia, MO, USA) and prepartum (−15 days) with Salmonella typhimurium bacterin-toxoid plus immune stimulator (Endovac-Bovi with immuneplus; Immvac, Columbia, MO, USA). Artificial insemination was performed using the FMS technique once between 34 and 41 days of pregnancy. The fetal membrane slip was detected by compression of the pregnant uterine horn and allowing the chorioallantoic membranes to slip between the fingers. All the palpation per rectum was performed by the same person, who avoided palpating the amniotic sac. Throughout the investigational period, the females did not undergo any other palpation per rectum. Both groups were submitted to two additional transrectal ultrasonographic examinations at days 45 and 60 of pregnancy. Day 45 was used to monitor the potential immediate deleterious effect of palpation per rectum on embryo viability [41], whereas day 60 was used to monitor the potential delayed deleterious effect of palpation per rectum on fetus viability [41]. All transrectal ultrasonographic examinations were performed in the morning by the same operator, using an ultrasound machine equipped with a 5 MHz linear transducer (Aloka 500 SSD, Corometrics Medical Systems, Inc., Wallingford, CT, USA). During the transrectal ultrasonography procedure, the operator

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Number of pregnant dairy cow/heifers in the palpated per rectum (PAL) and not palpated per rectum (NPAL) groups (controlled randomized block design)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total no. of females</td>
<td>PAL group (%)</td>
</tr>
<tr>
<td>520</td>
<td>258</td>
</tr>
<tr>
<td>By category</td>
<td></td>
</tr>
<tr>
<td>Cows (%)</td>
<td>360 (69.2)</td>
</tr>
<tr>
<td>Heifers (%)</td>
<td>160 (30.8)</td>
</tr>
<tr>
<td>By embryo no.</td>
<td></td>
</tr>
<tr>
<td>Singleton (%)</td>
<td>473 (91.0)</td>
</tr>
<tr>
<td>Twins (%)</td>
<td>47 (9.0)</td>
</tr>
</tbody>
</table>
removed the feces from the rectum, introduced the transducer, and avoided grasping the uterine horns. The transducer was cleaned between animals. The diagnosis of embryo/fetal death was made when there were no embryo/fetal heart beat, a lack of positive signs of pregnancy in an animal previously diagnosed pregnant, or the presence of signs of embryo/fetal degeneration.

The null hypothesis was that palpation per rectum using the fetal membrane slip technique for pregnancy diagnosis is not deleterious for the conceptus. The sample size required for a two-sided alternative hypothesis to be able to detect a difference of 10% between groups with a pregnancy loss (between days 29–32 and 60) of 10% using α-error of 5% and β-error of 0.20 was 219 pregnant animals for each group [42]. The proportion of animals suffering embryo/fetal death was compared between treatment groups using Chi-square analysis. A difference was considered significant at $P < 0.05$ [43].

### 3. Results

The overall rate of embryo/fetal death between days 30 and 60 was 14.0% (73/520; Table 2). Embryonic death (from days 30 to 45; 10.0%; 52/520) was higher than fetal death (from days 46 to 60; 4.5%; 21/468; $P < 0.001$). Embryo/fetal death was not different ($P > 0.20$) between the PAL group (14.7%; 38/258) and the NPAL group (13.4%; 35/262). Embryo death between PAL and NPAL groups was 9.3% (24/258) and 10.7% (28/262), respectively ($P > 0.20$). Fetal deaths between the PAL and the NPAL group were 5.9% (14/234) and 3.0% (7/234), respectively ($P > 0.20$). In the PAL group, the embryo mortality after palpation per rectum on days 34–37 was 16.5% (21/127) and for days 38–41 was 12.9% (17/131), respectively ($P > 0.05$). In cows, embryo/fetal death between PAL (18.6%; 33/177) and NPAL (14.2%; 26/183) was not different ($P > 0.10$). In heifers, embryo/fetal death, for the same groups was 6.2% (5/81) and 11.4% (9/79), respectively, which were not different ($P > 0.08$). Embryo/fetal mortality was higher in cows (16.4%; 59/360) than in heifers (8.8%; 14/160; $P < 0.025$). The percentage of twins for all pregnancies was 9.0% (47/520) and increased to 12.8% (46/360) when only cows were included. The percentage of twins for heifers (0.6%; 1/160) was different from the cows (12.8%; 46/360; $P < 0.001$). Embryo/fetal mortality was higher in twins (25.5%; 12/47) than in singleton pregnancies (12.9%; 61/473; $P < 0.025$). The number of twins for the PAL and NPAL groups was 23 and 24, respectively. Embryo/fetal mortality for twins was 21.7% (5/23) for the PAL group and 29.2% (7/24) for the NPAL group ($P > 0.10$).

### 4. Discussion

In the present study, the rate of spontaneous embryo/fetal mortality in early bovine pregnancy was not affected by the technique for pregnancy diagnosis using the fetal membrane slip. This experiment was designed to avoid the potential confounding factors found in previous reports, specifically: (1) only animals pregnant with a viable embryo were included; (2) a control group (not palpated) was included; (3) pregnant females were scheduled for subsequent TRUS evaluation to determine the potential immediate (day 45) and delayed (day 60) effect of PPR; (4) the percentage of animals pregnant with twins was equilibrated between groups; (5) animals that were sick/ill during the experimental period were eliminated from the study; (6) cows and heifers were analyzed separately; (7) the palpation per rectum was done with care.

Several other studies have dealt with the effect of palpation per rectum on embryo/fetal mortality. Some were retrospective [12,13] and others prospective [11]. In all of these studies, however, a non-palpated control was absent. This was, possibly, because at the time these studies were performed, a reliable alternative method for pregnancy diagnosis was not available.

In one study, early pregnancy diagnosis was associated with decreased diagnostic accuracy, increased embryo/fetal loss or increased calving interval [15]; however, there is no agreement from the results of another study [29]. In a retrospective study, cows diagnosed pregnant by experienced veterinarians at day 41 or less after insemination were significantly less likely to calve than were cows diagnosed pregnant later in pregnancy [15]. In Holstein cows from three dairies, prenatal losses were 5.8% for

### Table 2

<table>
<thead>
<tr>
<th>Embryo/fetal mortality in dairy cow/heifers in palpated per rectum (PAL) and not palpated per rectum (NPAL) groups</th>
</tr>
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<tbody>
<tr>
<td><strong>Total (%)</strong></td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>Total (%)</td>
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<tr>
<td>By category</td>
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<tr>
<td>Cows (%)</td>
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<tr>
<td>Heifers (%)</td>
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<tr>
<td>By embryo no.</td>
</tr>
<tr>
<td>Singleton (%)</td>
</tr>
<tr>
<td>Twins (%)</td>
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Within a column, rows without a common superscript differ: $^aP < 0.025$, $^bP < 0.01$. |
cows palpated at less than day 35, 6% for cows palpated between days 35 and 45, and 0.9% for palpations per rectum later than day 45 after AI [12]. Among 892 cows from 14 herds, apparent fetal loss was 8.5% for cows palpated 35 to 51 days post-breeding and 3.7% if palpation was done at days 52 to 70, estimated by palpation per rectum 30 to 90 days after the initial diagnosis [11]. A retrospective study of nearly 7500 Holstein and Guernsey cows found that embryonic loss measured by rebreeding or subsequent palpation per rectum was 7.2% among cows in the first 50 days after breeding, compared with 3.2% for cows palpated more than 50 days after breeding [13]. Although all three of these studies lacked a pregnant non-palpated group, the earlier the pregnancy diagnosis was done, the higher the embryo/fetal mortality. In the present study, only pregnant animals with a viable embryo and a non-palpated group were included. Thus, the effects of palpation per rectum and the effect of spontaneous embryo/fetal mortality during early stages of pregnancy were separated. In the non-palpated group of our study, embryo mortality was higher than the fetal mortality (10.7% versus 3.0%, respectively), consistent with some of the reports previously mentioned. Studies that used milk progesterone concentration as the indicator of pregnancy reported an embryo/fetal mortality of 10–28% from days 14 to 70 after insemination [44]. A recent study from California showed an embryo/fetal mortality of 19% between days 28 and 90 in lactating dairy cows [45].

The use of transrectal ultrasonography improved the reliability of the experimental design because only animals pregnant with a healthy embryo were included, and non-pregnant and pregnant animals with dead embryos were excluded. In addition, pregnant animals that, by palpation per rectum, would have been diagnosed open were included (i.e. reduction of false negatives) [33]. In previous studies, pregnancy diagnosis was assumed to be 100% accurate. In studies that used palpation per rectum as the method of diagnosis, the probability of false negative diagnosis was not tested (elimination of true pregnant females) or the inclusion of animals that were really non-pregnant (production of “false” pregnant females) was not ruled out. Some studies showed that palpation per rectum for pregnancy diagnosis was not an accurate procedure during the early stages of gestation. Two studies reported that 9 and 5% of the cows diagnosed open calved at a time consistent with being pregnant when the diagnosis was made [46,47]. Another study using only heifers showed that 9% of the animals detected pregnant by protein B at days 30–45 were not detected by palpation per rectum [20]. After spontaneous embryo/fetal death, the presence of positive signs of pregnancy (positive membrane slip) by palpation per rectum persists for some time despite an already dead embryo and cannot be determined precisely during early stages of embryo mortality [28,33]. In the present study, 15 pregnant females detected by TRUS were initially excluded from the experiment because of the presence of an embryo already dead, but exhibited positive fetal membrane slip. These animals presumably would have been “false positives” if palpation per rectum, progesterone concentration, or protein B had been used for the initial pregnancy diagnosis.

In the present study, 26.3% (10/38) of the females included in the PAL group presented negative signs of pregnancy (absence of fetal membrane slip) at the time of their assigned day of PPR. These animals were submitted to TRUS on that same day and confirmed not pregnant. This showed clearly that embryo death was not due to PPR per se. However, using TRUS on that same day, these animals were confirmed to be non-pregnant; this showed clearly that embryo death was not due to the PPR per se. However, these animals were not excluded from the statistical analysis. Conversely, the possibility that pregnant animals with a positive fetal membrane slip at the time of PPR in which the embryo was already dead or in the early process of degeneration was not tested and cannot be ruled out. In spontaneous embryo/fetal death, a positive fetal membrane slip persisted for 16.2 days (range, 7–28) [28], and similar values of 15.9 days (range, 7–27) [7], 16.3 days (range, 5–50) [9] and 18 days [23] were obtained when iatrogenic abortion by rupturing the amniotic vesicle was performed.

In this study, the palpation technique was probably similar to those used by most veterinarians in private practice; that is, each female was palpated per rectum once by a single veterinarian searching for fluid and a fetal membrane slip. In previous studies, realistic conditions were not followed because the animals were evaluated by more than one person at the same time, different techniques were used at the same time, or different techniques were used in the same animal by more than one person [11–14]. Therefore, all these studies utilized a more invasive palpation per rectum procedure than the one used in the present study for pregnancy diagnosis. The females studied through clinical teaching programs may have experienced more trauma as the result of less-experienced persons and/or more rigorous and extensive examination of the reproductive tract than what is typical in private practice. It is necessary to note that rupture of amniotic sac or
crushing the embryo/fetus by palpation per rectum can be used to cause embryo/fetal attrition and was frequently used for this purpose before the introduction of prostaglandin F2α [7–10]. This potential effect cannot be ruled out as a confounding variable on previous studies. According to some authors, the use of fetal membrane slip can harm the embryo/fetus [11]. However, this study included two trials and when the fetal membrane slip technique was used on one farm by only trained clinicians (first trial), no difference in embryo/fetal mortality was found compared with other techniques (fluctuation and amniotic sac palpation). Older reports consider fetal membrane slip a safe technique for early pregnancy diagnosis [5,6]. There are few studies comparing different techniques of palpation per rectum for early pregnancy diagnosis. Two of these reports found no difference between the fetal membrane slip and amniotic sac palpation [48,49]. It is important to realize that the present study was not designed to explore differences between techniques, because only one technique was used. However, the fetal membrane slip was shown not to produce damage in the embryo compared with the non-palpated pregnant control group.

Dairy cattle herds are affected by a multitude of management factors that influence calving rates [18]. Other studies have reported a herd effect on the relationship between palpation per rectum and embryo/fetal loss [17]; furthermore, there was a significant difference among herds in different years [13]. Many studies regarding palpation per rectum were combined with data collected from different farms. In the present study, only one farm with a consistent herd health management program was used.

Field data and review papers have documented large differences among breeds in twinning rates [50,51]. The twinning rate in Hereford and Angus breeds averages less than 1%, but in the Holstein breed exceeds 4% [50]. The induction of twin calving by transfer of one embryo day 7 after AI [52] or two embryos after unilateral or bilateral transfer [53] produced a high rate of embryo/fetal mortality [51]. Little information is available regarding embryo/fetal death in spontaneously occurring twins. Previous studies regarding mortality and palpation per rectum did not report the number of twin pregnancies [11,12]. Factoring twins into the results is important, as it can skew the results. In the present study, the number of twins was balanced between groups; the embryo/fetal mortality was twice that in singleton pregnancies.

Embryo/fetal mortality in cows was almost double that in heifers in the same stage of pregnancy. In most studies regarding palpation per rectum, heifers and cows were not separated [11,12]. A retrospective study using palpation per rectum between 30 and 70 days after breeding showed that embryo/fetal death was almost three times higher in cows than in heifers [30]. Although it is not well known why cows lose more embryos/fetuses than heifers, twinning rate could be a contributing factor. In cows which were induced to be pregnant with twins by transferring two embryos and then slaughtered at day 45, the numbers of placentomes were higher in the bilateral transfer than in the unilateral transfer [53]. Therefore, competition between conceptions in twin pregnancy for nutrition, space, or both could account for some of the pregnancy loss. In the present study, the percentage of twin pregnancies in was 0.6 and 12.8% in heifers and cows, respectively. When the numbers of cows with twins were excluded from the statistical analysis, a reduction in the percentage of embryo/fetal mortality was observed. However, embryo/fetal mortality continued to be higher in cows than heifers. Therefore, factors other than twinning rate are implicated in such embryo/fetal mortality. Stress of lactation, deficient nutritional support, and insufficient hormonal concentration, could account for this increased mortality rate.

In previous studies, the interval between palpation per rectum and re-evaluation was 30–90 days [11], 44–48 days [14] at calving [12], or variable depending if the palpation was performed in females before or after day 40 of pregnancy [13]. In the present study, all the females were scheduled for TRUS at days 45 and 60 of pregnancy. The rationale was to maintain an identical interval sufficient to determine the true association between palpation per rectum and embryo/fetal mortality and to exclude factors other than palpation per rectum that could affect pregnancy. The goal of the TRUS at day 45 was to determine an immediate effect of palpation per rectum during the embryo period, and the goal of the TRUS at day 60 was to determine a delayed effect of palpation per rectum manifested during the fetal period (from days 46 to 60) [41].

In conclusion, PPR for early pregnancy diagnosis in dairy cattle using the fetal membrane slip technique did not affect embryo/fetal viability.

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