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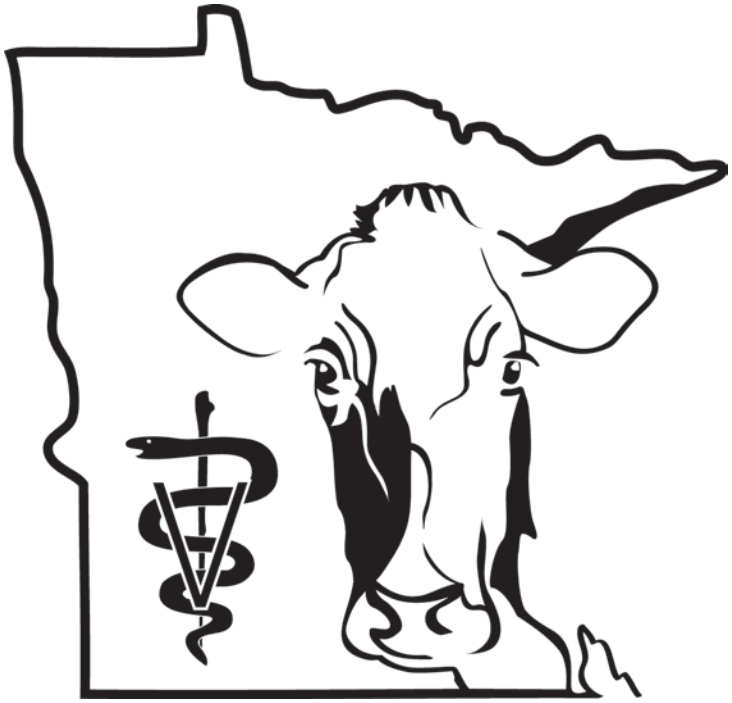

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Early Pregnancy Diagnosis by Palpation per Rectum on Embryo/Fetus Mortality in Dairy Cattle

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Introduction

In bovine practice, palpation per rectum is one of the most frequent methods used for pregnancy diagnosis by veterinarians after 30 days of breeding [Roberts, 1971; Momont, 1990; Youngquist, 1997]. It is considered that a trained practitioner is able to detect pregnant/non-pregnant animals by 35 days post-breeding [Zemjanis, 1971; Roberts, 1971; Momont, 1990; Youngquist, 1997; Euler, 1930; Götze, 1940]. The importance of a systematic and non-traumatic technique of palpation per rectum cannot be over emphasized as it is well known that embryonic/fetal deaths can be induced accidentally or iatrogenically by this procedure [Ball and Caroll, 1963; Dawson, 1974; Parmigiani et al., 1978].

An important step in dairy management is to examine for pregnancy/non-pregnancy within 45 days after breeding [Zemjanis, 1971]. The main purpose of examining cows early is not only to identify pregnant cows but also to identify with confidence open cows in order to manage, treat, cull or estrous synchronize them again [Zemjanis, 1971]. Early pregnancy diagnosis can assist dairymen in managing open cows and improving reproductive performance and economics of their herd [Oltenacu et al., 1990].

There is contradictory information about the potential deleterious effect of palpation per rectum for early pregnancy diagnosis on embryo/fetus viability. Some studies have suggested a possible adverse effect of early palpation per rectum [Abbitt et al., 1978; Paisley et al., 1978; Vaillancourt et al., 1979; Franco et al., 1987; White et al., 1989; McLeod and Williams, 1991]. In contrast, other recent studies [Thurmond and Picanso, 1993; Thompson et al., 1994] have suggested little effect of the time at which the first palpation per rectum is performed after insemination on calving rate. These studies had several flaws in their design. Previous reports that diagnosed pregnant females by palpation per rectum [Abbitt et al., 1978; Paisley et al., 1978; Vaillancourt et al., 1979], progesterone [Franco et al., 1987] or protein B [Alexander et al., 1995] did not assess the viability of the embryo/fetus. Most of the studies lack a "pregnant non-palpated group" [control group] [Abbitt et al., 1978; Paisley et al., 1978; Vaillancourt et al., 1979] to differentiate the effects of palpation per rectum from spontaneous embryo/fetal death occurring during early pregnancy. The interval between palpation per rectum and reevaluation was variable: from 30 to 90 days [Abbitt et al., 1978], 44 to 48 days [Franco et al., 1987], at calving [Paisley et al., 1978] or variable depending if the palpation was performed before or after 40 days of pregnancy [Vaillancourt et al., 1979]. This is important because the viability of the embryo/fetus can be affected by factors other than

palpation per rectum. High level of peripheral progesterone as indication of pregnancy is also not completely accurate. Progesterone level is high in conditions other than pregnancy, as presence of luteal cysts, long estrous cycles, sampling during luteal phase, pyometra [Pennington et al., 1976] as well as in a pregnant females with embryo/fetus death [Kassam et al., 1987]. Progesterone level is a better indicator of "non pregnancy" status than of pregnancy status [Shemesh et al., 1978; Laing et al., 1976]. The bovine pregnant female produces different kinds of specific proteins of pregnancy such as protein B. Bovine pregnancy specific protein B [bPSPB], a glycoprotein produced by the trophoblast, persists elevated despite embryo/fetus death or embryos in the process of degeneration [Maurer et al., 1985; Humblot et al., 1988]. In induced embryo/fetal death elevated levels of progesterone or protein B [Kassam et al., 1987] as well as positive signs of pregnancy persisted for several days despite the embryo/fetal death [Parmigiani et al., 1978; Kassam et al., 1987]. In a recent study, persistence of elevated levels or protein B was detected in spontaneous embryo/fetal mortality in dairy cattle [Romano, 2004]. Differences among farms in pregnancy loss are well established and are more related to management factors than to infectious diseases [Thompson et al., 1995]. This was not taken into consideration in some studies. Most of the previous reports pool together heifers with cows. One study showed that pregnant heifers have lower embryo/fetal mortality rates than cows [Labernia et al., 1996] however, these data were retrospective and without a control group. Previous studies did not report the number of twin pregnancies. Twin pregnancies increase the risk of embryo/fetal death and abortion [Day et al., 1995]. Moreover, in previous studies, real practice conditions were not followed. For example, females were palpated per rectum 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 [Abbitt et al., 1978; Paisley et al., 1978; Vaillancourt et al., 1979; Franco et al., 1987]. In the above cases, the procedure of palpation per rectum was more invasive than the one used for diagnosis of pregnancy in practice.

Confirmation of pregnancy status before or at the time of palpation per rectum by another method allows the differentiation of naturally occurring embryonic/fetal loss from embryonic/fetal loss potentially induced by palpation per rectum. The use of transrectal ultrasonography permits an earlier pregnancy diagnosis than palpation per rectum, gives immediate information about the presence of positive signs of pregnancy as well as on embryo/fetus viability and reduces the number of false positive diagnoses and false negatives when palpation per rectum is used [Romano and Magee, 2001]. 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 [Kähn, 1992; Ball and Logue, 1994; Baxter and Ward, 1997].

The objective of this presentation is to cover three studies: the first which evaluated the effects of palpation per rectum using the fetal membrane slip technique for early pregnancy diagnosis on pregnancy loss. The second study, which evaluated the degree of invasiveness of palpation per rectum on pregnant females on the proportion pregnancy loss. The third study compared the effect of palpation per rectum using the fetal membrane slip technique not only on proportion of pregnancy loss but also in the type of embryo/fetal death.

Materials and methods

First study:

Pregnant cows/heifers that developed clinical mastitis, severe degree of lameness or severe digestive disorders during the experimental period were excluded. The vaccination program included only products that contained killed bacteria and viruses. The time of vaccination for cows was scheduled at the following periods: at post-partum [25-30 days], vaccination 6 months later, at dry-off [-60 days] and pre-partum [-15 days].

A controlled randomized block design with two blocks one by category and the other by number of embryos were performed. The categories were cows and heifers and by the number of embryos were single and twin pregnancies. Five hundred and twenty pregnant females [360 cows and 160 heifers] with a viable embryo detected by transrectal ultrasonography between days 29 and 32 after artificial insemination were used. A viable embryo was defined as an embryo with heart rate above 120 beats per minute determined by counting the number of contractions/minute or by using M-mode of the ultrasound. The pregnant females were randomly divided in two equals groups: palpation per rectum [PAL group] and no palpation per rectum [NPAL group]. The PAL group was submitted to palpation per rectum using the fetal membrane slip [FMS] technique once between days 34 and 41 of pregnancy. All palpation per rectum was performed by the same person who avoided palpating the amniotic sac. Throughout the investigation period the females did not undergo any other palpation per rectum. Both groups were submitted to two additional transrectal ultrasonographies 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. Day 60 was used to monitor the potential delayed deleterious effect of palpation per rectum on fetus viability. All transrectal ultrasonographies were performed in the morning, by the same operator, using an Aloka SSD 500 ultrasound machine equipped with a 5 MHz linear transducer. During the transrectal ultrasonography procedure the operator removed the feces from the rectum, introduced the probe for scanning and avoided grasping the uterine horns. The probe was cleaned between animals. The diagnosis of embryo/fetal death was made when there was no embryo/fetus heart beat, signs of embryo/fetus degeneration were observed or when the positive signs of pregnancy were absent in a cow/heifer previously diagnosed as pregnant.

Second study

Four hundred and eighty three pregnant females with a viable embryo between 29 and 32 days detected by transrectal ultrasonography of pregnancy were used. The experimental design used was a controlled randomized block design as the first study. The females were divided in three groups: control [CON; n= 159], palpation per rectum 1 [PAL-1; n= 163] and palpation per rectum 2 [PAL-2; n=161]. The control group was not submitted to palpation per rectum. The PAL-1 and PAL-2 groups were submitted to palpation per rectum using the fetal membrane slip [FMS] technique once or twice between days 34 and 41 of pregnancy, respectively. These groups were submitted to two additional transrectal ultrasonographies at days 45 and 60 as reported in the first study. The same criteria of pregnancy and pregnancy loss as the first study were used.

Third study

In this study, 580 pregnant females with a viable embryo diagnosed by transrectal ultrasonography were used. Two hundred seventy-two females were palpated per rectum using the fetal membrane slip technique once between 34 and 41 days of pregnancy. Three hundred eight females were not palpated per rectum. These animals were evaluated again by transrectal ultrasonography at 45 and 60 days later as for studies 1 and 2. The use of palpation per rectum for embryo/fetus attrition produced a pregnancy loss characterized for persistence of positive fetal membrane slip by palpation per rectum [2 weeks], signs of conceptus degeneration by transrectal ultrasonography [3 weeks] and maintenance of a functional corpus luteum [4 weeks; Ball and Caroll, 1963; Parmigiani et al., 1978; Kassam et al., 1987]. Therefore, if palpation per rectum for pregnancy diagnosis is deleterious for the embryo/fetus the proportion of pregnancy loss should be higher than from a non-palpated pregnant group [positive control group] and second, the embryo/fetus mortality characterized for persistence of positive signs of pregnancy, signs of conceptus degeneration and persistence of a functional corpus luteum should be higher than the non-palpated pregnant group.

Statistical analysis

The proportion of animals suffering embryo/fetal death was compared between treatment groups using Chi-square analysis. A difference was considered statistically significant at $P<0.05$ [Devore and Peck, 1993].

Results

First study

The overall embryo/fetal death between days 30 and 60 was 14.0%. Embryonic death [from 30 to 45 days; 10.0%] was significantly higher than fetal death [from 46 to 60 days; 4.5%; $P<0.001$]. Embryo/fetus death between PAL group [14.7%] and NPAL group [13.4%] was not significantly different [$P>0.05$]. Embryo/fetus mortality was higher in cows [16.4%] than in heifers [8.8%; $P<0.025$]. Embryo/fetus mortality was higher in twins [25.5 %] than in single pregnancies [12.9%; $P<0.025$].

Second study

Pregnancy loss between days 30 and 60 of pregnancy was 12%, 13.5% and 13.0% for control, PAL-1 and PAL-2 groups, respectively [$P>0.05$]. No statistical differences were detected among groups in all the possible comparisons [$P>0.05$].

Third study

Out of 272 pregnant females in the palpation per rectum group, 41 were found with pregnancy loss [15.1%] while out of the 308 females pregnant in the non-palpation

per rectum group 46 were found with pregnancy loss [14.9%] at day 60 after artificial insemination [P>0.05]. Embryo/fetal death characterized for persistence of positive signs of pregnancy from the palpation per rectum group was 17 [6.3%] and 26 [8.4%] from the non-palpation per rectum group, respectively [P>0.05].

Discussion

The three previous studies show that palpation per rectum using the fetal membrane slip did not increase the pregnancy loss in pregnant females. The improvement in the experimental design was an important factor that permitted differentiation from "spontaneous" pregnancy losses to the possible effect of the palpation per rectum per se. The use of transrectal ultrasonography allowed the detection of pregnant females with a viable embryo was an approach that permitted a sound comparison from previous studies that used: palpation per rectum [Abbitt et al., 1978; Paisley et al., 1978; Vaillancourt et al., 1979; White et al., 1989; McLeod and Williams, 1991], progesterone in milk [Franco et al., 1987] or protein B in blood [Alexander et al., 1995]. This experimental design allowed separate the effect of palpation per rectum from the effect of period of diagnosis because a positive control group of non-palpated pregnant females was included. In addition, the use of transrectal ultrasonography eliminated pregnant females with embryo already dead that later will be found not pregnant.

The palpation per rectum technique used in all the present studies was probably similar to those used by most veterinarians in private practice, that is, each female was submitted to palpation per rectum by only one person once trying to find a positive sign of pregnancy. In previous studies, realistic conditions were not followed because the females were evaluated for 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 [Abbitt et al., 1978; Paisley et al., 1978; Vaillancourt et al., 1979; Franco et al., 1987].

Previous studies regarding pregnancy loss and palpation per rectum did not report the number of twin pregnancies. 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. Embryo/fetal mortality was twice in twin pregnancies compared with single pregnancies.

Pregnancy loss in cows was almost double that in heifers in the same stage of pregnancy. In most of the studies regarding palpation per rectum, heifers and cows were not separated [Abbitt et al., 1978; Paisley et al., 1978]. A retrospective study using palpation per rectum between 30 to 70 days after breeding showed that embryo/fetal death was almost three times higher in cows than in heifers [Labernia et al., 1996]. The reasons why cows lose more embryos/fetuses than heifers are unknown. Twinning rate can be one of these factors. However, in the present studies when the number of cows with twins were excluded from the statistical analysis a reduction in the percentage of embryo/fetal mortality was observed. Nevertheless, embryo/fetal mortality continued to be higher in cows than heifers. Therefore, factors other than twin pregnancy are implicated in such pregnancy loss. Stress of lactation, deficient nutritional support and insufficient hormonal levels, could be other causes for this increased mortality rate.

The use of more than one fetal membrane slip as measure of invasiveness of the technique did not increase the proportion of pregnancy loss. In addition, embryo/fetal

mortality was not different in the group with one fetal membrane slip compared to the positive control group. This showed that this technique did no present a deleterious effect on the conceptus under the present experimental conditions.

In summary, from these three studies it was concluded that early pregnancy diagnosis using fetal membrane slip technique did not increase the proportion of embryo/fetal death in dairy cattle.

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