

Is per rectum palpation for pregnancy diagnosis deleterious for the conceptus? New information about an old quandary

Juan E. Romano

Large Animal Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX

Introduction

In bovine practice, per rectum palpation of uterine contents is one of the most frequent methods used for pregnancy diagnosis by veterinarians around the world.¹⁻⁷ It is generally understood that a trained veterinarian is able to correctly detect pregnant and nonpregnant animals by days 35 after breeding.¹⁻¹¹ The importance of a systematic and nontraumatic technique of per rectum palpation (PRP) cannot be overemphasized, as it is well known that embryonic or fetal deaths can be induced accidentally or iatrogenically by PRP of the uterus.¹²⁻¹⁴

An important step in intensive cattle production systems (e.g., dairy farms, embryo transfer programs, and pure breed farms) is to perform a pregnancy diagnosis within 45 days after breeding.^{10,15} The main purpose of examining cows or heifers early after insemination is not only to correctly detect pregnant cows but also to detect with confidence nonpregnant females in order to manage, treat, cull, or synchronize estrus.^{10,16} Early pregnancy diagnosis can assist the producer in managing nonpregnant females and improving reproductive performance and economics of their herd.¹⁷

There is contradictory information about the potential deleterious effect of PRP of the uterus for early pregnancy diagnosis on embryo or fetus viability. Some studies have suggested an adverse effect of PRP.¹⁸⁻²³ In contrast, other studies²⁴⁻²⁶ have suggested little effect of the time at which the first PRP is performed after insemination on calving rate.

Previous investigators diagnosed pregnant females by PRP,¹⁸⁻²⁰ progesterone concentrations^{21,23} or pregnancy-specific proteins.^{26,27} However, these methods are not able to assess the viability of the embryo or fetus. Most published studies lack a positive control group of contemporaneous “pregnant non-per rectum palpated group”,¹³⁻¹⁵ to differentiate the effects of PRP from the spontaneous pregnancy loss occurring during early pregnancy. The interval between PRP and reevaluation varied from 30 to 90 days,¹³ 44 to 48 days,¹⁶ at calving¹⁴ or flexible depending if the PRP was performed before or after 40 days of pregnancy.¹⁵ A high level of peripheral progesterone as an indication of pregnant females is not a completely accurate method of pregnancy diagnosis. Progesterone level is high in conditions other than pregnancy, as in presence of luteal cysts, long estrous cycles, short estrous cycle, sampling during luteal phase, pyometra²⁸ as well as in a pregnant female with embryo or fetus death or in process of degeneration.^{15,29} Progesterone level is an excellent indicator of nonpregnancy status rather than pregnancy status.^{30,31} The pregnant bovine female produces different kinds of PSP such as protein B. Bovine pregnancy-specific protein B (bPSP-B), a glycoprotein produced by the giant cells of the trophoblast, however, remains elevated despite death or degeneration of the embryo or fetus.^{15,27,32} In induced embryo/fetal death, elevated levels of progesterone²⁹ or PSP-B,²⁷ as well as positive signs of pregnancy, persisted for several days despite the embryo or fetal death.^{14,29} In a recent study of spontaneous pregnancy loss in dairy cattle, elevated levels of PSP-B persisted¹⁵

Differences among farms in pregnancy loss are well established and are related more to management factors than to infectious diseases.³³ This was not taken into consideration in some studies. Most of the previous investigations pool heifers with cows. One study showed that pregnant heifers have lower pregnancy loss than cows;³⁴ however, these data were retrospective and lacked a control group. Twin pregnancies increase the risk of pregnancy loss.^{7,35} Interestingly, in most of the studies dealing with pregnancy loss, the number of twin pregnancies was not reported.⁷

Studies in the United States and in other areas of the world have found an association between amniotic sac per rectum palpation (ASP) during the embryonic period for pregnancy diagnosis until day 45 of gestation³⁶ and an increased risk of atresia coli/jejuni in newborn calves.³⁷⁻⁴¹ In atresia coli/jejuni, a section of the large bowel or jejunum is absent, resulting in a blind intestinal tube. This clinical congenital condition is lethal, and surgical correction is the only treatment available.⁴²⁻⁴⁵ Atresia

coli/jejuni has been reported in different countries and in more than ten breeds of cattle, with a marked predominance in Holstein calves.⁴⁶ Based on those findings, some authors have recommended avoiding PRP of the uterus during the first 45 days of gestation.^{36-39,47,48} Intestinal atresia was also reported to be inherited as an autosomal-recessive trait in Jersey and Swedish Highland cattle.^{49,50} Intestinal atresia could develop either from imperfect canalization, of the gut or from insufficient blood supply to the affected portion of the intestine⁵¹ and ASP was suggested to act for this latter mechanism.^{39,46} Nevertheless, the cause of atresia coli/jejuni remains controversial and not completely understood.^{45,46,51} In the author's practice, ASP is not routinely used for early pregnancy diagnosis; however, cases of atresia coli or jejuni were detected. Interestingly, cases of atresia coli/jejuni were also diagnosed in newborn calves from dams that underwent PRP only by detection of allantochorion membrane during the first trimester of gestation, either during the late embryonic period or fetal period, during the second trimester of gestation; females were diagnosed as pregnant only by transrectal ultrasonography (TRUS; Romano, unpublished data).

Finally, in most of these previous studies, real practice conditions for PRP were not followed; that is only one veterinarian palpated per rectum each cow or heifer once looking for positive signs of pregnancy. In those studies, females were palpated by more than one person successively or different techniques were used in the same animal by more than one person¹³⁻¹⁶ In those cases, the procedure of PRP appears more invasive than that used for pregnancy diagnosis in a regular practice.

Confirmation of pregnancy status before or at the time of PRP by another method allows for the differentiation of spontaneous pregnancy loss from pregnancy loss potentially induced by PRP. The use of transrectal ultrasonography permits an earlier pregnancy diagnosis than PRP, gives immediate information about the presence of positive signs of pregnancy as well as embryo or fetus viability, and reduces the potential misdiagnosis of pregnancy.^{11,52} In addition, reports about the use of ultrasonography during pregnancy have shown that it is a safe technique that does not affect embryo or fetus viability.⁵³⁻⁵⁷

The objective of this manuscript is to cover five studies, the first of which evaluated the effects of PRP using the fetal membrane slip technique for early pregnancy diagnosis during late embryonic period on pregnancy loss. The second study evaluated the degree of invasiveness of PRP on pregnant females on the proportion of pregnancy loss. The third study compared the effect of PRP using the fetal membrane slip technique, not only on proportion of pregnancy loss but also in the type of pregnancy loss. The fourth study evaluated the effect of detection of ASP during the late embryonic period on pregnancy loss. The fifth study assessed the effect of detection of ASP during the late embryonic period for pregnancy diagnosis on pregnancy loss, calving rates, and abnormalities in newborn calves.

Materials and methods

First study

A controlled randomized block design with two blocks, one by category and the other by number of embryos, was performed.⁷ The categories were cows and heifers. Single and twin pregnancies were determined based on number of embryos. Five hundred and twenty clinically healthy pregnant females (360 cows and 160 heifers) with a viable embryo detected by transrectal ultrasonography between days 29 and 32 after artificial insemination were included. The pregnant females were randomly divided into two equal groups: per rectum palpation (PAL group) and no per rectum palpation (NPAL group). The PAL group was subjected to PRP using the fetal membrane slip (FMS) technique (detection of allantochorion membrane) once between days 34 and 41 of pregnancy.⁵⁸ All PRPs were performed by the same person who avoided palpating the amniotic sac. Throughout the investigation period the females did not undergo any other PRP. Both groups were subjected to additional transrectal ultrasonography at days 45 and 60 of pregnancy. Day 45 was used to monitor spontaneous late embryonic death for the control group and the potential immediate deleterious effects of PRP on embryo viability. Day 60 was used to monitor spontaneous early fetal death for the control group and the potential late deleterious effect of PRP on viability of the fetus. All TRUS procedures were performed in the morning, by the same operator, using an Aloka SSD 500 ultrasound machine equipped with a 5 MHz linear transducer. The diagnosis of

pregnancy loss was made when there was no embryonic or fetal heart beat; signs of embryonic/fetal degeneration were observed, or when the positive signs of pregnancy were absent in a cow or heifer previously diagnosed as pregnant by TRUS.

Second study

A controlled randomized block design was performed.⁵⁹ The blocking was performed by farms, categories, and number of embryos. Nine hundred twenty eight pregnant females with a viable embryo between 28 and 34 days detected by TRUS were included. Two farms were used. The categories were cows and heifers. The number of embryos determined single and twin pregnancies. The females were divided in two groups: control (CON; n= 476) and per rectum palpation (PAL; n= 452). In addition, the PRP groups were randomly divided in per rectum palpation 1 (PAL-1; n= 230) and per rectum palpation 2 (PAL-2; n=222). The control group was not subjected to PRP. The PAL-1 and PAL-2 groups were subjected to PRP using the FMS technique once or twice at the same examination between days 34 and 43 of pregnancy, respectively. Further, these groups were subjected to two additional TRUS procedures at days 45 and 60 as described in the first study and the same criteria of pregnancy and pregnancy loss were used.

Third study

In this study, 580 pregnant females with a viable embryo diagnosed by TRUS (as studies 1 and 2) were used.⁶⁰ Two hundred seventy-two females were palpated using the FMS technique once between 34 and 41 days of pregnancy, and 308 females not palpated were included. These animals were evaluated again by TRUS at 45 and 60 days later as in studies 1 and 2. The use of PRP for embryonic or fetal attrition (induced abortion) produced a pregnancy loss that is characterized by persistence of a positive FMS detected by PRP for two weeks, signs of conceptus degeneration for three weeks detected by TRUS (three weeks), and maintenance of a functional corpus luteum for around four weeks^{12,14,29} as described for spontaneous pregnancy loss type I.¹⁵ Therefore, if PRP for pregnancy diagnosis is deleterious for the embryo or fetus, the proportion of pregnancy loss should be higher than in a nonpalpated pregnant group (positive control group), and the pregnancy loss will be characterized by persistence of positive signs of pregnancy, signs of conceptus degeneration, and persistence of a functional corpus luteum and should be higher than the non-per rectum palpated pregnant group.

Fourth study

A controlled randomized block design that included 347 clinically healthy lactating pregnant cattle was performed.⁶¹ All cattle were detected pregnant by use of TRUS at approximately day 29 after estrus and randomly allocated into two groups (control group [CON; n = 167 cows] and amniotic sac per rectum palpation group [ASP; n= 180]). The CON group was not subjected to pregnancy diagnosis via PRP. The ASP group involved the compression of the pregnant uterine horn by PRP and detection of the amniotic sac vesicle between the hand and the fingers.⁶² The entire ASP was performed by one trained veterinarian between days 34 and 43 after estrus. All cattle were reevaluated by TRUS on days 45, 60, and 90 to determine viability of conceptus. The same criteria of pregnancy and pregnancy loss were used as in the previous studies.

Fifth study

The present investigation was a randomized controlled blocked double-blind design that included 680 pregnant lactating dairy cows.⁶³ The blocks for farms and two farms (farms A and B) were used. From each farm, the females were diagnosed as pregnant based on the presence of a viable embryo by TRUS between Days 28 and 45 after breeding by the same veterinarian. Then, the pregnant females were randomly assigned to control (CON group, no PRP) and treatment groups (ASP group, per rectum amniotic sac palpation). The CON group did not receive any PRP of the uterus. All the ASPs were performed only once between Days 34 and 45 after artificial insemination by a board-certified theriogenologist. After being subjected to their respective treatments, each female was reevaluated for

pregnancy only by TRUS again between two and four weeks later 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. In farm B, pregnancy reexamination was performed by two different veterinarians who were blind to the treatment but were aware about the project. Then, all of the females pregnant at reexamination were followed until calving. Every abortion, premature calf, or stillbirth was submitted for necropsy to determine the type of abnormalities, if any. The veterinarians in charge of the necropsy were different from the initial PRP and from the reexaminations. All the calves born alive were maintained for observation for three to five days postpartum to detect any of abnormalities.

Statistical analysis

The proportion of females suffering pregnancy loss was compared between treatment groups using Chi-square analysis or Fisher exact test as appropriate. In addition, binary logistic regression analysis was used to detect the effect of farm (second and fifth study), categories (first, second and third studies) and the number of embryos (first, second, third, and fourth studies). A difference was considered statistically significant at $P < 0.05$.^{64,65} A software program was used to analyze all data sets.⁶⁶

Results

First study

The overall pregnancy loss between days 30 and 60 was 14.0%. Late embryonic pregnancy loss (from 30 to 45 days; 10.0%) was significantly higher than fetal death (from 46 to 60 days; 4.5%; $P < 0.001$). Pregnancy loss between the PAL group (14.7%) and the NPAL group (13.4%) was not significantly different ($P > 0.05$). Pregnancy loss was higher in cows (16.4%) than in heifers (8.8%; $P < 0.025$) and in females pregnant with twins (25.5 %) compared with single pregnancies (12.9%; $P < 0.025$).

Second study

The overall percent of pregnancy loss between days 28-34 and 60 was 14.1% (131/928). Late embryonic pregnancy loss for CON, PAL-1 and PAL-2 groups was 12.6% (60/477), 9.1% (21/230), and 9.5% (21/221), respectively ($P = 0.28$). Fetal pregnancy loss for the same groups was 2.4% (10/417), 3.8% (8/209), and 5.5% (11/200), respectively ($P = 0.15$). The overall percentage of pregnancy loss for CON, PAL-1, and PAL-2 from days 28 to 34 to 60 was 14.7% (70/477), 12.6% (29/230), and 14.5% (32/221), respectively ($P = 0.74$). No statistical differences were detected among groups in all the possible comparisons.

Third study

Out of 272 pregnant females in the PRP group, 41 were found with pregnancy loss (15.1%), while out of the 308 females pregnant in the non-PRP group, 46 were found with pregnancy loss (14.9%) at day 60 after artificial insemination ($P > 0.05$). Pregnancy loss characterized by persistence of positive signs of pregnancy was 17 (6.3%) from the PRP group and 26 (8.4%) from the non-PRP group, respectively ($P > 0.05$). No statistical differences between groups either in the proportions and type of pregnancy loss were detected.

Fourth study

Overall pregnancy loss between days 29 and 90 was 12.7% (44/347). Overall pregnancy loss for late embryonic period (days 29 to 45; 9.5%) was significantly ($P = 0.001$) higher than for early (days 46 to 60; 2.5%) or late (days 61 to 90; 1%) fetal periods. The pregnancy loss between days 29 and 90 between CON and ASP groups was 13.2% and 12.2%, respectively ($P = 0.79$). Late embryonic pregnancy loss for the CON and ASP groups was 10.8% and 8.3%, respectively ($P = 0.44$). Early fetal pregnancy loss for CON and ASP groups was 1.3% and 3.6%, respectively ($P = 0.93$), and late fetal pregnancy loss for the

same groups was 1.4% and 0.6%, respectively (P=79). No differences were detected between early and late fetal period (P=0.24). The percentage of cows with twins at initial pregnancy diagnosis by TRUS was 7.5%. The pregnancy loss (days 29 to 90) in single pregnancies was 12.2%, and in twin pregnancies, it was 19.2% (P=0.29).

Fifth study

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

In farm A, calving rate one for the CON group was 81.2% (134/165), and it was 80.8% (147/182; P=0.92) for the ASP group; calving rate two for the same groups was 92.4% (134/145) and 94.8% (147/155), respectively (P=0.68). In farm B, calving rate one for the CON group was 77.7% (132/170), and it was 74.8% (119/159; P = 0.55) for the ASP group; calving rate two for the same groups was 87.4% (132/151) and 82.1% (119/145), respectively (P=0.20). The calving rate one for farm A was 80.9% (281/347), and it was 75.4% (251/333; P=0.14) for farm B; calving rate two for farm A was 92.4% (281/304), and it was 84.8% (251/296; 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.

Discussion

These five independent studies showed that per rectum palpation for pregnancy diagnosis using either the FMS or amniotic sac detection did not increase the pregnancy loss.^{7,59-61,63} The experimental design allowed differentiation between the effects of PRP from the spontaneous pregnancy losses, because a positive control group of contemporaneous pregnant females non-per rectum palpated was included. This approach was only possible at this time, because TRUS permitted detection of pregnant females and the assessment of conceptus viability at an earlier stage of pregnancy. In addition, TRUS excluded pregnant females with an embryo already dead or in process of degeneration from all these investigations.^{7,52,59-61,63}

In the current studies, the PRP technique used was similar to those employed by most veterinarians in private practice for pregnancy diagnosis. Each female was subjected to PRP by only one person a single time while trying to find a positive sign of pregnancy. In earlier reports, realistic conditions were not followed, because the females were evaluated by more than one person successively; different techniques were utilized, or various procedures were applied successively to the same female by more than one person.¹⁸⁻²¹

Previous investigators of PRP and pregnancy loss did not report the number of twin pregnancies. Factoring twins into the result is important, as it can skew the results.^{7,15,59} In some of the present studies, the numbers of twins were balanced between groups; therefore, the high inherent risk of pregnancy loss of twin pregnancies was equalized between treatments. The risk of pregnancy loss is more than double in twin pregnancies compared with single pregnancies.^{7,15,59}

Pregnancy loss in cows was almost double than in heifers at the same stage of pregnancy. In most previous studies of PRP, heifers and cows were not separated.^{18,19} A study, in which PRP was used between 30 and 70 days after breeding, showed that pregnancy loss was almost three times higher in cows

than in heifers.³⁴ The reasons why cows lose more pregnancies than heifers are unknown. Twinning rate can be one of these factors. However, in the present experiments, when the number of cows with twins was excluded from the statistical analysis a reduction in the percentage of pregnancy loss was observed. Nevertheless, pregnancy loss continued to be higher in cows than heifers. Therefore, factors other than twin pregnancy are implicated in such pregnancy loss. Stress of lactation, subclinical mastitis, deficient nutritional support, and insufficient hormonal levels could be other causes for this increased mortality rate.

All of the studies agreed that examinations during the embryonic period detected more spontaneous pregnancy loss compared with later periods.^{7,59-61,63} Therefore, it is recommended that any female detected as pregnant during late embryonic period will require a further reexamination to reduce the chances of potentially maintaining a nonpregnant female in the production system.

In the second study, the use of more than one FMS at the same examination for pregnancy diagnosis as a measure of invasiveness of the technique did not increase the proportion of pregnancy loss.⁵⁹ The proportion of pregnancy loss was not different among the groups, with one or two fetal membrane slips at the same examination compared to the positive control group of non-palpated per rectum pregnant females. These results showed that PRP was not deleterious for the conceptus under the same experimental conditions.

The third investigation not only confirmed the results of the first two studies, but also supports with additional evidence that PRP was not harmful to the conceptus.⁶¹ If PRP produced embryonic or fetal attrition, the pregnancy loss would be characterized by persistence of positive signs of pregnancy by per rectum palpation, signs of embryo/fetus degeneration and functional corpus luteum by TRUS.^{12-15,29} Consequently, this type of pregnancy loss would be significantly higher compared with the positive control group of non-per rectum palpated pregnant females. However, no difference in the proportion of this type of pregnancy loss between treatments in this investigation was detected, therefore, showing with this new approach that PRP was not detrimental for the conceptus.

The fourth experiment showed that ASP for pregnancy diagnosis was a safe procedure for the conceptus compared with the CON group, because no increase in pregnancy loss in the three periods of assessment was detected.⁶¹ The three check points at the end of the embryonic period (day 45), early fetal period (day 60), and late fetal period (day 90) were designed to estimate the potential immediate, late, and delayed effect of ASP on pregnancy loss, respectively. This new study showed that by using a different positive sign of pregnancy, such as the amniotic sac detection, was not harmful for the pregnancy as it was also demonstrated when allantochorion membrane detection was used. This investigation adds new information and expands our knowledge that when using either allantochorion membrane or amniotic sac detection, no increase in pregnancy loss was observed.

The fifth study showed no increase of pregnancy loss in the ASP group compared with the CON group and is in agreement with the fourth report.⁶³ In the current study, the two calves with atresia coli were diagnosed only in the CON group, which is in contrast to the previous studies that associated ASP during the late embryonic period with atresia coli or atresia jejuni.³⁷⁻⁴⁸ The ASP group included 341 pregnant females that underwent PRP between days 34 and 45 and from which 285 were assessed between days 35 and 42 of pregnancy, which was considered the period of highest risk of producing atresia coli. Based on the prevalence of atresia coli from previous studies, it was expected in the present study that there would be between nine and 32 calves with atresia coli in the ASP group; however, no cases of atresia coli were produced. This is strong evidence against the deleterious effect of ASP on the conceptus.

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)^{10,36} for technical and economic reasons.^{7,10,17} 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 knowledge will not only affect the way that the veterinarians practice but also how the owner or manager will perceive the use of this procedure for reproductive management. Some authors previously recommended avoiding PRP of the uterus during the first 45 days of gestation.^{30,37-39,41,42,47,48} However,

the present investigation supports the theory that ASP for pregnancy diagnosis, when performed by a trained veterinarian, was a safe procedure for the conceptus using the three assessment points: at reexamination, calving, and evaluation of newborn calves.

In summary, from these five independent investigations, it was concluded that per rectum palpation for early pregnancy diagnosis using either the FMS technique or ASP did not increase the proportion of pregnancy loss, decrease the calving rates, or increase the abnormalities in newborn dairy calves. In addition, an increased risk of spontaneous pregnancy loss was detected in cows versus heifers, during late embryonic period compared with the fetal period, in females carrying twins as opposed to singletons, and differences in pregnancy loss among farms were also noticed.

References

1. Roberts SJ: Veterinary obstetrics and genital diseases. Ithaca(NY): Published by the author; 1971.
2. Momont H: Rectal palpation: safety issues. *Bov Pract* 1990;25:122-123.
3. Youngquist RS: Pregnancy diagnosis. In: Youngquist RS, editor. *Current therapy in large animal theriogenology*. Philadelphia: WB Saunders Co; 1997. p. 295-303.
4. Vandeplassche M: Reproductive efficiency in cattle: a guideline for project in developing countries. Rome, Italy. F.A.O. No 25; 1982.
5. USDA Dairy. Part IV: Reference of dairy cattle health and management practices in the United States; 2007.
6. Cowie AT: Pregnancy diagnosis tests: a review. *Commonwealth Agricultural Bureaux Joint Publication, Number 13*; 1948. p. 11.
7. Romano JE, Thompson JA, Kraemer DC, et al: Early pregnancy diagnosis by palpation per rectum: influence on embryo/fetal viability in dairy cattle. *Theriogenology* 2007;67:486-493.
8. Euler V: Die klinischen Erkennungsmerkmale der Frühgraviditat beim Rind und ihre Verwertbarkeit für die Praxis. *Berl Tierärztl Wschr* 1930;30: 477-481.
9. Götze R: Die Feststellung der Schwangerschaft beim Rinde. *Dtsch Tierarztl Wschr* 1940;48:183-185.
10. Zemjanis R: Diagnostic and therapeutic techniques in animal reproduction. Baltimore: The Williams and Wilkins Co; 1962.
11. Romano JE, Fahning ML: Accuracy of per rectum palpation for pregnancy diagnosis in cattle. *Proc XXVIII World Buiatrics Congress*; 2014. p. 68.
12. Ball L, Carroll EJ: Induction of fetal death in cattle by manual rupture of the amniotic vesicle. *J Am Vet Med Assoc* 1963;142:373-374.
13. Dawson FLM: Methods for early termination of pregnancy in the cow. *Vet Rec* 1974;94:542-548.
14. Parmigiani E, Ball L, Lefever D, et al: Elective termination of pregnancy in cattle by manual abortion. *Theriogenology* 1978;10:283-290.
15. Romano JE: Early pregnancy diagnosis and embryo/fetal mortality in cattle [dissertation]. College Station: Texas A and M University; 2004.
16. Romano JE, Thompson JA, Forrest DW, et al: Early pregnancy diagnosis by transrectal ultrasonography in dairy cattle. *Theriogenology*, 2006;66:1034-1041.
17. Oltenacu PA, Ferguson JD, Lednor AJ: Economic evaluation of pregnancy diagnosis in dairy cattle: a decision analysis approach. *J Dairy Sci* 1990;73: 2826-2831.
18. Abbitt B, Ball L, Kitto GP, et al: Effect of three methods of palpation for pregnancy diagnosis per rectum on embryonic and fetal attrition in cows. *J Am Vet Med Assoc* 1978;73:973-977.
19. Paisley LG, Mickelson WD, Trost OL: A survey of the incidence of prenatal mortality in cattle following pregnancy diagnosis by rectal palpation. *Theriogenology* 1978;9:481-491.
20. Vaillancourt D, Bierschwal CJ, Ogwu D, et al: Correlation between pregnancy diagnosis by membrane slip and embryonic mortality. *J Am Vet Med Assoc* 1979;175:466-468.
21. Franco OJ, Drost M, Thatcher MJ, et al: Fetal survival in the cow after pregnancy diagnosis by palpation per rectum. *Theriogenology* 1987;27:631-644.
22. White ME, LaFaunce N, Mohammed HO: Calving outcomes for cows diagnosed pregnant or nonpregnant by per rectum examination at various intervals after insemination. *CanVet J* 1989;30:867-870.
23. McLeod BJ, Williams ME: Incidence of ovarian dysfunction in post partum dairy cows and the effectiveness of its clinical diagnosis and treatment. *Vet Rec* 1991;128:121-124.
24. Thurmond MC, Picanso JP: Fetal loss associated with palpation per rectum to diagnose pregnancy in cows. *J Am Vet Med Assoc* 1993;203:432-435.
25. Thompson JA, Marsh WE, Calvin JA, et al: Pregnancy attrition associated with pregnancy testing by rectal palpation. *J Dairy Sci* 1994;77:3382-3387.
26. Alexander BM, Johnson MS, Guardia RO, et al: Embryonic loss from 30 to 60 days post breeding and the effect of palpation per rectum on pregnancy. *Theriogenology* 1995;43:551-556.
27. Humblot P, Camous S, Martal J, et al: Pregnancy-specific protein B, progesterone concentrations and embryonic mortality during early pregnancy in dairy cows. *J Reprod Fertil* 1988;83:215-223.

28. Pennington JA, Spahr SL, Lodge JR: Factors affecting progesterone in milk for pregnancy diagnosis in dairy cattle. *Brit Vet J* 1976;132:487-495.
29. Kassam A, BonDurant RH, Basu S, et al: Clinical and endocrine responses to embryonic and fetal death induced by manual rupture of the amniotic vesicle during early pregnancy in cows. *J Am Vet Med Assoc* 1987;191:417-420.
30. Shemesh M, Ayalon N, Shalev E, et al: Milk progesterone measurement in dairy cows: correlation with estrus and pregnancy determination. *Theriogenology* 1978;9:343-353.
31. Laing JA, Gibbs HA, Eastman SAK: A herd test for pregnancy in cattle based on progesterone levels in milk. *Brit Vet J* 1976;132:204-209.
32. Maurer RR, Ruder CA, Sasser RG: Effectiveness of the protein B radioimmunoassay to diagnose pregnancy in beef cattle[abstract]. *J Anim Sci* 1985;61(Suppl 1):390.
33. Thompson JA, Marsh WE, Etherington WG, et al: Evaluation of the benefits of the timing of pregnancy testing by transrectal palpation in dairy cattle. *J Am Vet Med Assoc* 1995;207:1462-1465.
34. Labernia J, Lopez-Gatius F, Santolaria P, et al: Influence of management factors on pregnancy attrition in dairy cattle. *Theriogenology* 1996;45:1247-1253.
35. Day JD, Weaver LD, Franti CE: Twin pregnancy diagnosis in Holstein cows: discriminatory powers and accuracy of diagnosis by transrectal palpation and the outcome of twin pregnancies. *Can Vet J* 1995;36:93-97.
36. Committee on Bovine Reproductive Nomenclature. Recommendations for standardizing bovine reproductive terms. *Cornell Vet* 1972;62:216-237.
37. Bellows RA, Rumsey TS, Kasson CW, et al: Effects of organic phosphate systemic insecticides on bovine embryonic survival and development. *Am J Vet Res* 1975;36:1113-1140.
38. Ness VH, Leopold G, Müller W: Zur Genese des angeborenen Darmverschlusses (atresia coli et jejuni) des Kalbes. *Mh Vet Med* 1982;37:89-92.
39. Müller W, Kelker L, Wünsche K, et al: Derzeitiger Stand der Ermittlungen zum Vorkommen und Zur Ätiologies angeborenen Darm verschlusses bei Kälbern im Bezirk Dresden. *Mh Vet-Med* 1982;37:84-89.
40. Schlegel F, Müller W, Willer S, et al: Die rektale Frühträchtigkeitsuntersuchung als auslösender Faktor der partiellen Kolonaplasie beim Rind. *Mh Vet-Med* 1986;41:377-382.
41. Brenner J, Orgad U: Epidemiological investigations of an outbreak of intestinal atresia in two Israeli dairy herds. *J Vet Med Sci* 2003;65:141-143.
42. Ducharme NF, Arighi M, Horney D, et al: Colonic atresia in cattle: a prospective study of 43 cases. *Can Vet J* 1988;29:818-824.
43. Constable PD, Rings DM, Hull BL, et al: Atresia coli in calves; 26 cases (1977-1987). *J Am Vet Med Assoc* 1989;195:118-123.
44. Smith DF, Ducharme NG, Fubini SL, et al: Clinical management and surgical repair of atresia coli in calves: 66 cases (1977-1988). *J Am Vet Med Assoc* 1991;199:1185-1190.
45. Constable PD, Huhn JC, Morin DE, et al: Atresia coli in calves: etiopathogenesis and surgical management. *Bov Pract* 1999;33:70-73.
46. Constable PD, Shanks RD, Huhn J, et al: Evaluation of breed as a risk factor for atresia coli in cattle. *Theriogenology* 1997;48:775-790.
47. Bellows RA, Patterson DJ, Burfering PJ, et al: Occurrence of neonatal and postnatal mortality in range beef cattle. II. Factors contributing to calf death. *Theriogenology* 1987;28:573-586.
48. Ducharme N, Gilbert R, Smith DF: Atresia coli: genetics or iatrogenics. *Proc Annu Meet Soc Therio*; 1990. p 112-117.
49. Jubb KVP, Kennedy PC, Palmer N: Pathology of domestic animals. Vol 2. 4th ed. New York: Academic Press; 1992.
50. Nihleen B, Eriksson K. A hereditary lethal defect in calves – atresia ilei. *Nord Vet Med* 1958;10:113-127.
51. Johnson R. Intestinal atresia and stenosis: a review comparing its etiopathogenesis. *Vet Res Comm* 1986;10:95-104.
52. Romano JE, Magee D: Applications of trans-rectal ultrasonography in cow/heifer reproduction. In: Annual Food Conference. Conception to Parturition: Fertility in Texas Beef Cattle. College of Veterinary Medicine. Texas A & M University; 2001. p. 99-104.
53. Reece EA, Assimkopoulos E, Zheng XZ, et al: The safety of obstetric ultrasonography: concern for the fetus. *Obstet Gynecol* 1990;76:139-146.
54. Kähn W. Ultrasonography as a diagnostic tool in female animal reproduction. *Anim Reprod Sci* 1992;28:1-10.
55. Ball PJH, Logue DDN: Ultrasound diagnosis of pregnancy in cattle. *Vet Rec* 1994;34:532.
56. Baxter SJ, Ward WR: Incidence of fetal loss in dairy cattle after pregnancy diagnosis using a ultrasound scanner. *Vet Rec* 1997;140:287-288.
57. Miller DL. Safety assurance in obstetrical ultrasound. *Semin Ultrasound CT MR* 2008;29:156-64.
58. Abelein V. Frühdiagnose der Gravidität beim Rind. *Münch Tierärztl Wschr* 1928;79:4.
59. Romano JE, Thompson JA, Kraemer DC, et al: Early pregnancy diagnosis by palpation per rectum on pregnancy loss in dairy cattle. *J Am Vet Med Assoc* 2011;239:668-673.
60. Romano JE. Further evidence that per rectum palpation for early pregnancy diagnosis is not harmful for the conceptus. In preparation
61. Romano JE, Fahning ML: Effects of early pregnancy diagnosis by palpation of amniotic sac per rectum on pregnancy loss in dairy cattle. *J Am Vet Med Assoc* 2013;243:1462-1467.
62. Pissl V: Zur Frühdiagnose der Trächtigkeit beim Rind. *Tierärztliche Rundschau* 1923;29:370-371.

63. Romano JE, Bryan K, Ramos R, et al: Effect of early pregnancy diagnosis by per rectum amniotic sac palpation on pregnancy loss, calving rates, and abnormalities in newborn dairy calves. *Theriogenology* 2016;85:419-427.
64. Devore J, Peck R. *Statistics: the exploration and analysis of data*. 2nd ed. Belmont(CA): Duxbury Press , 1993.
65. Hulley SB, Cummings SR, Browner GD, editors: *Designing clinical research: an epidemiologic approach*. 2nd ed. Philadelphia: Lippincott Williams & Wilkins; 2001.
66. Minitab 16: Minitab Inc. State College, PA

