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Reproductive performance of dairy cows resynchronized after pregnancy diagnosis at 31 (±3 days) after artificial insemination (AI) compared with resynchronization at 31 (±3 days) after AI with pregnancy diagnosis at 38 (±3 days) after AI

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Abstract

An important part of reproductive management programs on dairy farms is identification of nonpregnant cows and early re-insemination to achieve higher pregnancy rates. The objective of this study was to compare the effect on reproductive performance and pregnancy loss of 2 pregnancy diagnosis protocols: (1) pregnancy diagnosis performed 31 ± 3 d after artificial insemination (AI) by ultrasonography (ULTRA), and (2) resynchronization started 31 ± 3 d after AI but with pregnancy diagnosis performed 38 ± 3 d after AI by palpation per rectum (PALP). Cows were randomly allocated into 1 of the 2 management programs. For cows enrolled in ULTRA, the initial pregnancy diagnosis (P1) was performed by transrectal ultrasonography at 31 ± 3 d after AI, and nonpregnant cows were enrolled in the Ovsynch protocol for resynchronization of ovulation to receive timed AI (TAI). For cows enrolled in PALP, the Ovsynch protocol for resynchronization of ovulation to receive TAI was initiated at 31 ± 3 d after AI regardless of pregnancy status, with the initial pregnancy diagnosis (P1) performed by palpation per rectum at 38 ± 3 d after AI. For both groups, reconfirmation of pregnancy was performed by palpation per rectum at 63 ± 3 d after AI (P2). Cows were inseminated after detection of estrus by use of activity monitors at any time during the study. Two levels of activity were used as a reference for cows AI after detection of estrus based on activity: an activity level of ≥2 when a cow was coded in DairyComp 305 (Valley Agricultural Software, Tulare, CA) as open (nonpregnant) and an activity level of ≥3 when the pregnancy status of the cow was unknown. Our findings showed that the odds of pregnancy loss cows in ULTRA was 2 times higher between P1 and P2 compared with that of cows in PALP. Furthermore, pregnancy diagnosis method (ULTRA vs. PALP) did not have a significant effect on the Cox proportional hazard of pregnancy at P2. The occurrence of assisted parturition, metritis, or retained placenta was associated with a reduced hazard of pregnancy at P2. An economic analysis was performed by simulating a 1,000-cow commercial dairy herd using a decision support tool to estimate the net present value (NPV; $/ cow per yr) from using the 2

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different pregnancy diagnosis methods. The analysis revealed minor differences in NPV between the programs, depending on the cost to perform ULTRA or PALP. In summary, we observed no difference in the reproductive performance and only a minor and fluctuating economic difference when using either PALP or ULTRA for pregnancy diagnosis of dairy cows.

Keywords

pregnancy; ultrasonography; palpation per rectum; Ovsynch

INTRODUCTION

Pregnancy diagnosis is an important component of reproductive management programs, where efficient and accurate pregnancy diagnosis combined with proper interventions determine the success of the reproductive programs and ultimately dairy farm profitability (Gröhn and Rajala-Schultz, 2000; Inchaisri et al., 2010).

The number of days a cow is nonpregnant after the voluntary waiting period has been associated with reduced profitability partly because of increased breeding cost, increased risk of culling, replacement cost, and reduced lifetime milk production (De Vries, 2006). To reduce losses associated with days nonpregnant, early identification of cows that fail to conceive can be used to obtain greater economic gains from reproductive programs in dairy herds (Fricke, 2002).

Palpation per rectum is currently the method most frequently used by veterinarians for pregnancy diagnosis, and skilled practitioners are able to detect pregnancy by palpation per rectum in cattle as early as 35 d after insemination (Romano et al., 2007). The use of transrectal ultrasonography has traditionally been considered an ancillary technology for identification of pregnancy alone in dairy cattle, and its use has largely been limited to occasions when identification of cows carrying twin fetuses, detection of ovarian and uterine disease, or determination of fetal sex is required (Hanzen et al., 2000; Fricke, 2002). However, the use of ultrasonography for pregnancy diagnosis is quickly becoming a common practice.

A veterinary-grade ultrasound machine equipped with one rectal transducer can be purchased for $8,000 to $16,000 (Fricke, 2002). A survey of pregnancy diagnosis practices used for dairy cows by US veterinarians revealed that the median charge for pregnancy diagnosis per cow was $3 when using palpation per rectum and $4.75 when using ultrasonography (Rosenbaum and Warnick, 2004). The ability to accurately identify nonpregnant cows as early as 28 days after AI when using ultrasonography has a positive effect on reproductive performance by reducing the interval between AI services for cows diagnosed nonpregnant, resulting in greater economic net return (Nation et al., 2003; Romano et al., 2006; Giordano et al., 2013). On the other hand, extensive late embryonic mortality is observed in dairy cattle, with rates of spontaneous pregnancy losses between approximately 30 and 45 d of gestation of 0.85% per day (Vasconcelos et al., 1997; Chebel et al., 2003; Santos et al., 2004b). Therefore, performing early pregnancy diagnosis (e.g., at 31 d after AI) using ultrasonography could appear to result in a greater pregnancy loss at the time of a second pregnancy diagnosis (e.g., after 2 mo) compared with performing the first pregnancy diagnosis a week later (at 38 d) using palpation per rectum (Cartmill et al., 2001; Chebel et al., 2003; Santos et al., 2004b).

A commonly used resynchronization program consists of administering an injection of GnRH to all cows regardless of their pregnancy status 1 wk prior to pregnancy diagnosis. Once pregnancy diagnosis is performed, nonpregnant cows receive an injection of $PGF_{2\alpha}$ the same day, and 48 h later receive a second injection of GnRH and timed AI (**TAI**) 20 to 24 h later (Fricke et al., 2003). A disadvantage of resynchronization TAI protocols is the labor and drug cost for the first injection of GnRH that is administered to cows later diagnosed as pregnant. Furthermore, a reproductive management practice aimed at reducing the interbreeding interval in cows has been the combined detection of estrus with protocols for resynchronization of ovulation, which has been reported to have economic advantages, especially for reproductive programs with lower conceptions rates (Tenhagen et al., 2004; Giordano et al., 2011; Galvão et al., 2013).

The objective of this study was to compare the effect on reproductive performance and pregnancy loss of 2 pregnancy diagnosis protocols: pregnancy diagnosis performed 31 ± 3 d after AI by ultrasonography compared with resynchronization started 31 ± 3 d after AI but with pregnancy diagnosis performed 38 ± 3 d after AI by palpation per rectum.

MATERIALS AND METHODS

Farm and Management

Data were collected from a dairy farm in Cayuga County, New York, from July to December 2009. The farm milked 2,850 Holstein cows 3 times a day, with a daily average of 40 kg of milk per cow per day during the study period. Cows were housed in freestall barns with concrete stalls covered with mattresses and bedded with recycled manure solids. Cows were milked 3 times daily, with the first milking starting around 0600 h, the second at around 1400 h, and the third at around 2200 h. Cows were fed a TMR consisting of approximately 55% forage (corn silage, alfalfa and grass haylage, alfalfa silage, alfalfa hay, and wheat straw) and 45% concentrate (corn meal, soybean meal, canola meal, cotton seed, and citrus pulp). The diet was formulated to meet or exceed NRC (2001) requirements for lactating Holstein cows weighing 650 kg and producing 40 kg of 3.7% FCM (NRC, 2001).

Data Collection and Study Design

For this study, a randomized field trial was designed by enrolling lactating cows and replacement heifers of breeding age that may calve during the duration of the study. Randomization was completed in Excel (Microsoft, Redmond, WA) using the random number function and imported into the farm’s Dairy Comp 305 software program (Valley Agricultural Software, Tulare, CA). Before the beginning of the trial, a total of 4,945 animal (2,850 lactating cows, 473 dry cows, and 1,622 heifers within breeding age) were randomly assigned once to 1 of 2 reproductive management programs using 2 different pregnancy diagnosis methods (**PDM**): (1) the initial pregnancy diagnosis (**P1**) was performed by transrectal ultrasonography at 31 ± 3 d after AI, and nonpregnant cows were enrolled in the

Ovsynch protocol for resynchronization of ovulation to receive TAI (**ULTRA**); or (2) the Ovsynch protocol for resynchronization of ovulation to receive TAI was initiated at 31 ± 3 d after AI regardless of pregnancy status, with P1 performed by palpation per rectum at 38 ± 3 d after AI (**PALP**; Figure 1). A total of 1,590 cows were enrolled in the study, with 813 cows enrolled in ULTRA and 777 cows enrolled in PALP. Cows diagnosed pregnant at P1 had a second pregnancy diagnosis (**P2**) at 63 ± 3 d after AI by palpation per rectum. Data from cows that were sold or died before P2 were excluded from the analysis.

Three veterinarians from Cornell Ambulatory and Production Medicine Clinic (Ithaca, NY) performed all pregnancy diagnoses. One of the veterinarians had more than 25 yr of experience and used only palpation per rectum for pregnancy diagnosis. The remaining 2 veterinarians had more than 5 yr of experience performing pregnancy diagnosis in dairy cows and used both transrectal ultrasonography and palpation per rectum. Transrectal ultrasonography was performed with a portable ultrasound machine fitted with a 5-MHz linear array transducer. Pregnancy diagnosis was performed weekly, with cows being separated from their pen mates by using an automatic sorting gate, restrained by head locks or palpation rail, and examined for pregnancy according to the group to which they were assigned.

The reproductive management program for second and subsequent AI service at this farm consisted of a combination of detection of estrus solely by use of activity monitors (Alpro, DeLaval, Kansas City, MO), and resynchronization of ovulation for TAI for cows failing to conceive, not detected in estrus, and detected as nonpregnant at the time of pregnancy diagnosis. Estrus detection was solely based on electronic activity monitors placed around the neck. Cows were classified as having an activity level of 0, 1, 2, or 3, and we defined 2 different minimal activity level thresholds for AI. The activity level threshold for triggering AI in cows with unknown pregnancy status (from AI until the time of P1 according to the PDM group) was ≥3, whereas the threshold for cows that were not pregnant and not AI (cows between the end of voluntary waiting period and first AI or cows confirmed nonpregnant) was ≥2. The use of 2 levels of activity for estrus detection was a preference of the farm's reproductive management. Cows scheduled to undergo TAI were separated by an automatic sorting gate when exiting the first milking of the day, and cows tagged to undergo AI based on estrus detection were separated by an automatic sorting gate when exiting the milking subsequent to detection of estrus.

Cows diagnosed nonpregnant at P1 for ULTRA were enrolled in the Ovsynch protocol consisting of an initial dose of 100 μg of GnRH (Cystorelin, Merial Ltd., Duluth, GA) following the nonpregnancy diagnosis, followed 7 d later by 25 mg of $PGF_{2\alpha}$ (Lutalyse, Pfizer, New York, NY); 48 h later, cows received a second injection of 100 μg of GnRH and TAI 20 to 24 h later. Cows detected in estrus by the activity monitoring system were artificially inseminated and removed from the TAI list. Cows in the PALP group were enrolled in a resynchronization (Resynch) TAI protocol consisting of an initial injection of 100 μg of GnRH 7 d before the first pregnancy diagnosis, and cows diagnosed as nonpregnant at 38 ± 3 d after AI were injected with 25 mg of $PGF_{2\alpha}$ (Lutalyse, Pfizer). Forty eight hours later, cows received a second injection of 100 μg of GnRH and TAI 20 to

24 h later. For both ULTRA and PALP, cows diagnosed nonpregnant at second pregnancy diagnosis at 63 ± 3 d after AI were enrolled in the Ovsynch protocol to receive TAI.

Case Definitions

Pregnancy loss was defined as a pregnant diagnosis at 31 ± 3 d after AI (ULTRA) or at 38 ± 3 d after AI (PALP) followed by a nonpregnant diagnosis at P2 (63 ± 3 d after AI) or detection of estrus by the activity monitoring system after P1. Retained placenta was defined as a cow having failed to release its fetal membranes within 24 h of calving. Metritis was defined as the presence of a fetid, watery, brownish uterine discharge and the cow presenting a rectal temperature above 39°C. Assisted parturition was defined as the use of any manual or mechanical intervention to assist a cow during parturition.

Statistical Analysis

To evaluate the effect of PDM on the overall hazard of pregnancy, hazard of pregnancy for cows diagnosed nonpregnant and rebred, and hazard of pregnancy loss, 3 Cox semiparametric proportional hazard (**PH**) models were created in SAS (SAS Institute Inc., Cary, NC) by using the proportional hazard regression procedure. For the calculation of hazard of pregnancy for all AI and the hazard of pregnancy for cows diagnosed non-pregnant and rebred, the initial points for the Cox PH model were the initial AI before enrollment and P1, respectively. The outcome endpoint was a pregnant diagnosis at P2 with cows right-censored if not diagnosed pregnant before culling, death, coded in DairyComp 305 as “do not breed”, or the end of the data collection period. The Cox PH model for all AI only used the data for cow re-inseminations based on estrus detection when this event occurred after enrollment of the cow in the study.

In the Cox PH model for hazard of pregnancy loss, the initial point was a pregnant diagnosis at P1 and the outcome endpoint was a nonpregnant diagnosis at P2 or detection of estrus by activity monitors after enrollment; cows were right-censored if culling, death, or the end of the data-collection period occurred prior to the outcome endpoint. The PH assumptions were tested and met by using the Schoenfeld residuals test and the marginal residuals test (Allison, 2012). Kaplan-Meier survival analyses were used to illustrate the results from each of the 3 Cox PH models by using Medcalc version 11.5.1.0 (MedCalc Software, Mariakerke, Belgium). The survival analysis periods and censoring for the Kaplan-Meier survival analysis were the same as used for each of the 3 corresponding Cox PH models.

To evaluate the effect of PDM on the overall hazard of pregnancy throughout the study period, a Cox PH was fitted to the data using the PH regression procedure in SAS (SAS Institute Inc.). Variables offered to the models included PDM (ULTRA vs. PALP), parity (1 vs. 2 vs. >2), assisted parturition, metritis, and retained placenta. Two-way interactions between treatment and all independent variables were tested. The PH assumption was tested using the Schoenfeld residuals test and the marginal residuals test (Allison, 2012).

Because the Cox PH models could not determine the effect of AI service method (i.e., TAI vs. AI after detection of estrus) as cows may have been bred by activity in one breeding and by TAI in another breeding, logistic regression models were fitted to the data using the

Logistic procedure of SAS, allowing assessment of the effect of PDM on the odds of AI based on detection of estrus by activity monitors. The model included the fixed effect of PDM (ULTRA vs. PALP) and parity (1 vs. 2 vs. >2). To assess the effect of pregnancy diagnosis on the odds of conception for cows in ULTRA or PALP at P2, mixed logistic regression models were fitted to the data using the GLIMMIX procedure of SAS (SAS Institute Inc.). The model included the fixed effects of PDM (ULTRA vs. PALP), type of AI (TAI vs. activity detection), and a 2-way interaction between PDM and type of AI. To assess the effect of pregnancy diagnosis on the odds of pregnancy loss detected at P2, mixed logistic regression models were fitted to the data using the GLIMMIX procedure of SAS (SAS Institute Inc.). The model included the fixed effects of PDM (ULTRA vs. PALP), parity (1 vs. 2 vs. >2), and type of AI (TAI vs. activity detection), and a 2-way interaction between PDM and type of AI was tested. Cow identification number was added to the model as a random effect to account for cows participating in the study more than once. All statistical models, variables, and their interactions were considered significant when their respective P-values were ≤ 0.05.

Economic Analysis

To assess the effect of PDM on the reproductive dynamics and economics of a dairy operation, a 1,000-cow commercial dairy herd was simulated using the UW-DairyRepro$ decision support tool (Giordano et al., 2011) with the modifications described in Giordano et al. (2013) to account for the differences in pregnancy loss between the 2 PDM. Briefly, this tool accounts for the additional pregnancy loss observed during the period elapsed between an earlier and a later nonpregnancy diagnosis test (i.e., in the current study, the 7-d period between the test in ULTRA performed at 31 d and PALP performed at 38 d after AI). Cows undergoing pregnancy loss after P1 were detected in estrus according to a user-defined probability (service rate) and were re-inseminated immediately, whereas cows not detected in estrus were found nonpregnant at P2 within 4 wk after P1 and re-inseminated after synchronization of ovulation. Of significance to the present study, the tool accounts for the added cost of giving injections to cows that are pregnant in Resynch programs initiated before the nonpregnancy diagnosis (e.g., the PALP group in the present study). The reproductive program simulated for first AI service was similar to that used at the dairy farm where the study was performed (combination of detection of estrus with the Presynch-Ovsynch-12 protocol), whereas the 2 reproductive management programs compared in the present study combining resynchronization of ovulation initiated at 31 ± 3 d after AI with ULTRA or PALP in combination with detection of estrus were simulated for second and subsequent AI service. The proportion of cows receiving AI after detection of estrus was set at 50% with pregnancy per AI (**P/AI**) of 32%, whereas P/AI was set at 35 and 26% for first and second or subsequent TAI services, respectively. Total pregnancy loss during the 7-d period from 31 to 38 d after AI for the program using ULTRA was set to represent the additional pregnancy loss observed between PDM methods in this study. Reproductive program costs included GnRH at $2.50/dose, $PGF_{2\alpha}$ at $2.00/dose, labor for hormone injections at $15.00/h, and AI (includes semen unit and labor) at $15.00/AI. Nonpregnancy diagnosis cost for P1 was set at $95.00/h when using palpation per rectum and at $135.00/h when using ultrasonography. In addition, the simulated scenarios included productive (12,700 kg of milk of rolling herd average, 31% culling and mortality rate, and 6% stillbirth rate) and economic parameters (price of milk = $18/cwt, feed cost of lactating cows = 0.26 $/kg, feed cost of dry cows = $2.50/cow per d, female calf value = $200/calf, male calf value = $50/ calf, replacement heifer = $1,500/heifer, salvage value of culled cow = $700/ cow) commonly observed under the current economic conditions for a high-producing herd of similar size in the northeastern United States. The model estimated the net present value (**NPV**; $/ cow per yr) differences for the reproductive programs using the 2 PDM.

RESULTS

Descriptive Statistics

A total of 1,590 cows were enrolled in the study, with 777 in PALP and 813 in ULTRA; average DIM at enrollment was 108 for cows in PALP and 106 for cows in ULTRA. A total of 7% of cows in PALP and 8% of cows in ULTRA had a history of assisted parturition, 10% of cows in PALP and 8% of cows in ULTRA had a history of retained placenta, and 20% of cows in PALP and 19% of cows in ULTRA had a history of metritis. The percentage of cows in first, second, and third or greater parities were 39, 35, and 26%, respectively, for cows in PALP, and 36, 35, and 29%, respectively, for cows in ULTRA.

Effect of PDM on Hazard of Pregnancy

The overall reproductive performance was not significantly different between cows in PALP and ULTRA; the median number of days from the initial AI (last AI before enrollment) to conception (confirmed at P2) was 42 and 39 d for cows in PALP and ULTRA, respectively (P = 0.53; Figure 2). No effect of PDM on the hazard of pregnancy was observed (hazard ratio = 0.97, 95% CI: 0.86–1.08, P = 0.57). The factors assisted parturition, metritis, and retained placenta impaired reproductive performance (Table 1). The reproductive performance of cows that were diagnosed as nonpregnant at P1 and then re-inseminated did not differ(P = 0.35) between PALP and ULTRA; the median number of days from the initial AI to conception was 87 and 80 d for cows in PALP and ULTRA, respectively (P = 0.35; Figure 3).

Effect of PDM on Pregnancy Loss

For both PDM, most of the pregnancy losses were detected at P2 (P = 0.03; Figure 4). Cows enrolled in ULTRA had a 2 times greater odds for pregnancy loss compared with cows in PALP (P = 0.01; Table 2). Furthermore, cows that conceived from a re-insemination based on estrus detection were at 1.96 times higher odds of embryonic mortality compared with cows that conceived from TAI (P = 0.01; Table 2). An interaction between PDM and type of AI was observed (P = 0.007; Table 2). The highest incidence of pregnancy loss was observed for cows in ULTRA that were inseminated after estrus detection (15.6%), whereas the lowest incidence was observed for cows in PALP that received TAI (4.5%); parity did not affect pregnancy loss (P = 0.48, Table 2).

Effect of PDM on Observed Conception Rate and Estrus Detection

The PDM was not associated with conception rate at P1 or P2 (P = 0.46; Table 3). A 1.8 and 1.9 higher odds for conception at P1 and P2, respectively, were observed for AI based on detection of estrus by activity monitors compared with TAI (P = 0.06; Table 3). Cows re-inseminated in ULTRA had 2.5 times greater odds (95% CI: 1.33–4.67, $P = 0.004$) for AI based on detection of estrus by activity monitors compared with cows re-inseminated in PALP.

An interaction between PDM and type of AI was observed at P1 ($P = 0.05$; Table 3) but not at P2 ($P = 0.13$; Table 3). Cows in ULTRA re-inseminated based on activity monitors after P1 had a conception rate of 48.5% at the following P1, whereas cows in ULTRA that were re-inseminated based on TAI had a conception rate of 26.1% at the following P1. Cows in the PALP group that were re-inseminated based on activity monitors after P1 had a conception rate of 20% at the following P1, whereas cows in PALP that received TAI had a conception rate of 26.8% at P1 (Table 3).

Economic Analysis

Under the initial conditions stipulated in the simulation study for a 1,000-cow herd using ULTRA or PALP as the PDM with a 4.6-percentage-point (9.8% for ULTRA vs. 5.2% for PALP) greater pregnancy loss from 31 to 38 d after AI for the program using ULTRA, the NPV was \$3.65/cow per year greater for the program using PALP. Nevertheless, this small benefit in favor of the program using PALP was eliminated by reducing the cost to perform ultrasonography to \$115/h and reverted to \$3.65/cow per year in favor of the program that used ULTRA when the cost of ultrasonography was further reduced to \$95/h (same as the cost of palpation per rectum).

DISCUSSION

We did not observe a significant difference in reproductive performance when using ULTRA for early pregnancy diagnosis (at 31 ± 3 d after AI) compared with using PALP at a later time (38 ± 3 d after AI; Figure 2). Pregnancy loss between P1 and P2 was 9.8% for ULTRA and 5.2% for PALP. Most pregnancy losses in dairy cows occur before the period of maternal recognition of pregnancy around 18 to 25 d of gestation, although substantial losses continue to occur up to 56 d after AI (Santos et al., 2004b; Mamo et al., 2011). In commercial dairy farms, pregnancy diagnosis is commonly performed at 3 times during gestation: the first is usually performed between 28 and 45 d after insemination, the second between 60 and 120 d after insemination, and the third at dry-off. Embryonic mortality rates between the first and second pregnancy diagnoses have been reported to range between 10.5 and 17.7% (López-Gatius et al., 2002; Silke et al., 2002; Gümen et al., 2003; Giordano et al., 2012). Most of this mortality occurs at 28 to 45 d of gestation, with rates ranging from 3.2 to 11.4% of all recognized pregnancies (Vasconcelos et al., 1997; Silke et al., 2002; Santos et al., 2004a; Giordano et al., 2012). As expected, higher embryonic mortality was observed for the ULTRA group because the first pregnancy diagnosis was performed 7 d before that in the PALP group, which allowed for greater detection of naturally occurring embryonic mortality. Nevertheless, cows that experienced pregnancy loss in the ULTRA group tended to not be re-inseminated until after the second pregnancy diagnosis when they were enrolled in Ovsynch to receive TAI immediately.

In our study, most cows in the PALP group were inseminated following enrollment in the resynchronization TAI protocol at 31 ± 3 d after AI, with conception rates of 26.4% at P1 and 20.1% at P2 (Table 3). Moreira et al. (2000) observed similar findings when using a resynchronization TAI protocol starting at 20 d after AI, with a reported conception rate of 20% at 45 d of gestation. Fricke et al. (2003), when evaluating pregnancy per AI using a resynchronization TAI protocol, observed P/AI values of 23, 34, and 38% for cows enrolled in the resynchronization TAI protocol at 19, 26, and 33 d after AI, respectively. Furthermore, our results are in agreement with those of Silva et al. (2009), who enrolled cows in a resynchronization TAI protocol 32 d after AI and observed P/AI of 29.1 and 25.7% at 39 and 62 d after AI, respectively.

Several risk factors are known to be associated with reduced fertility and embryonic losses in cattle, including environmental stresses, disease, nutrition, luteal insufficiency, and ovulation of persistent follicles (Silke et al., 2002; Chebel et al., 2004; Santos et al., 2004b). Risk factors presenting a negative effect on hazard of pregnancy at P2 in our study were parturition, metritis, and retained placenta. Previous research has shown that cows with a history of dystocia have close to 2 times greater odds of having a retained placenta, which increases the occurrence of postpartum diseases and subsequently decreases reproductive performance in dairy herds (Han and Kim, 2005). Konyves et al. (2009), when studying postpartum uterine disease in dairy cows on subsequent reproduction, observed that cows with a history of retained placenta had 27 times greater odds of developing metritis. Uterine infections such as metritis contribute to reduced fertility by various means. Uterine infection has direct effects on the uterus through bacterial infection, bacterial products, and immune mediators produced in response to bacterial infection (Bicalho et al., 2010; Santos and Bicalho, 2012). Additionally, uterine diseases also suppress pituitary luteinizing hormone secretion and are associated with inhibition of folliculogenesis, decreased ovarian steroidogenesis, and abnormal luteal phases (Opsomer et al., 2000; Salasel et al., 2010; Crowe and Williams, 2012). By disrupting ovarian function, metritis contributes to reduced fertility in dairy cows and increases the likelihood of a cow being culled for reproductive failure.

In agreement with the lack of differences in reproductive performance for cows enrolled in ULTRA and PALP, only minor and fluctuating economic differences were observed. The small benefits in favor of the program using PALP were explained, for the most part, by the greater cost to perform ultrasonography in ULTRA. Indeed, reducing the hourly cost of ultrasonography services either eliminated or reverted the differences in NPV between the PDM. When the cost of ultrasonography was equal to that of palpation, the benefit in favor of ULTRA was likely due to the added cost of giving GnRH to pregnant cows in the strategy using palpation. To a lesser extent, the original differences in favor of PALP may have been due to the shorter time to re-insemination in cows that lose their pregnancy from 31 to 38 d after AI in PALP. Unlike cows undergoing pregnancy loss enrolled in PALP (receive TAI within 3 d of the nonpregnancy diagnosis), cows undergoing pregnancy loss enrolled in ULTRA in our simulation had a 50% chance of re-insemination if detected in estrus before P2 or received TAI after re-enrollment in the Ovsynch program when diagnosed nonpregnant at P2. The magnitude of the difference, however, was minimal because of the low percentage (4.6%) of additional cows with pregnancy loss that had delayed re-insemination in ULTRA versus PALP. Our results are in agreement with a previous simulation study using the same decision support tool, which reported that the additional pregnancy loss observed with an earlier pregnancy diagnosis method around 32 d after AI did not cause major economic losses (Giordano et al., 2013).

CONCLUSIONS

A significantly higher incidence of pregnancy loss was detected in ULTRA compared with PALP, but earlier detection and rebreeding of nonpregnant cows occurred in ULTRA, although the difference was not significant. Overall, our study did not observe a statistical difference in the reproductive performance of lactating dairy cows when using either PALP or ULTRA for pregnancy diagnosis.

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References

Allison, PD., editor. Survival Analysis Using SAS: A Practical Guide. 2. SAS Institute Inc; Cary, NC: 2012.

Bicalho RC V, Machado S, Bicalho ML, Gilbert RO, Teixeira AG, Caixeta LS, Pereira RV. Molecular and epidemiological characterization of bovine intrauterine Escherichia coli. J Dairy Sci. 2010; 93:5818–5830. [PubMed: 21094754]

Cartmill JA, El-Zarkouny SZ, Hensley BA, Lamb GC, Stevenson JS. Stage of cycle, incidence, and timing of ovulation, and pregnancy rates in dairy cattle after three timed breeding protocols. J Dairy Sci. 2001; 84:1051–1059. [PubMed: 11384031]

Chebel RC, Santos JE, Cerri RL, Galvao KN, Juchem SO, Thatcher WW. Effect of resynchronization with GnRH on day 21 after artificial insemination on pregnancy rate and pregnancy loss in lactating dairy cows. Theriogenology. 2003; 60:1389–1399. [PubMed: 14519461]

Chebel RC, Santos JE, Reynolds JP, Cerri RL, Juchem SO, Overton M. Factors affecting conception rate after artificial insemination and pregnancy loss in lactating dairy cows. Anim Reprod Sci. 2004; 84:239–255. [PubMed: 15302368]

Crowe MA, Williams EJ. Triennial Lactation Symposium: Effects of stress on postpartum reproduction in dairy cows. J Anim Sci. 2012; 90:1722–1727. [PubMed: 22064736]

De Vries A. Economic value of pregnancy in dairy cattle. J Dairy Sci. 2006; 89:3876–3885. [PubMed: 16960063]

Fricke PM. Scanning the future—Ultrasonography as a reproductive management tool for dairy cattle. J Dairy Sci. 2002; 85:1918–1926. [PubMed: 12214984]

Fricke PM, Caraviello DZ, Weigel KA, Welle ML. Fertility of dairy cows after resynchronization of ovulation at three intervals following first timed insemination. J Dairy Sci. 2003; 86:3941–3950. [PubMed: 14740830]

Galvão KN, Federico P, De Vries A, Schuenemann GM. Economic comparison of reproductive programs for dairy herds using estrus detection, timed artificial insemination, or a combination. J Dairy Sci. 2013; 96:2681–2693. [PubMed: 23415521]

Giordano JO, Fricke PM, Cabrera VE. Economics of resynchronization strategies including chemical tests to identify nonpregnant cows. J Dairy Sci. 2013; 96:949–961. [PubMed: 23261387]

Giordano JO, Fricke PM, Wiltbank MC, Cabrera VE. An economic decision-making support system for selection of reproductive management programs on dairy farms. J Dairy Sci. 2011; 94:6216–6232. [PubMed: 22118110]

Giordano JO, Wiltbank MC, Guenther JN, Pawlisch R, Bas S, Cunha AP, Fricke PM. Increased fertility in lactating dairy cows resynchronized with double-Ovsynch compared with Ovsynch initiated 32 d after timed artificial insemination. J Dairy Sci. 2012; 95:639–653. [PubMed: 22281329]

Gröhn YT, Rajala-Schultz PJ. Epidemiology of reproductive performance in dairy cows. Anim Reprod Sci. 2000; 60–61:605–614. [PubMed: 22422014]

Gümen A, Guenther JN, Wiltbank MC. Follicular size and response to Ovsynch versus detection of estrus in anovular and ovular lactating dairy cows. J Dairy Sci. 2003; 86:3184–3194. [PubMed: 14594238]

Han IK, Kim IH. Risk factors for retained placenta and the effect of retained placenta on the occurrence of postpartum diseases and subsequent reproductive performance in dairy cows. J Vet Sci. 2005; 6:53–59. [PubMed: 15785124]

Hanzen C, Pieterse M, Scenczi O, Drost M. Relative accuracy of the identification of ovarian structures in the cow by ultrasonography and palpation per rectum. Vet J. 2000; 159:161–170. [PubMed: 10712804]

Inchaisri C, Jorritsma R, Vos PL, van der Weijden GC, Hogeveen H. Economic consequences of reproductive performance in dairy cattle. Theriogenology. 2010; 74:835–846. [PubMed: 20580069]

Konyves L, Szenci O, Jurkovich V, Tegzes L, Tirian A, Solymosi N, Gyulay G, Brydl E. Risk assessment of postpartum uterine disease and consequences of puerperal metritis for subsequent metabolic status, reproduction and milk yield in dairy cows. Acta Vet Hung. 2009; 57:155–169. [PubMed: 19457784]

López-Gatius F, Santolaria P, Yaniz J, Rutllant J, Lopez-Bejar M. Factors affecting pregnancy loss from gestation day 38 to 90 in lactating dairy cows from a single herd. Theriogenology. 2002; 57:1251–1261. [PubMed: 12013445]

Mamo S, Mehta JP, McGettigan P, Fair T, Spencer TE, Bazer FW, Lonergan P. RNA sequencing reveals novel gene clusters in bovine conceptuses associated with maternal recognition of pregnancy and implantation. Biol Reprod. 2011; 85:1143–1151. [PubMed: 21795669]

Moreira F, Risco CA, Pires MF, Ambrose JD, Drost M, Thatcher WW. Use of bovine somatotropin in lactating dairy cows receiving timed artificial insemination. J Dairy Sci. 2000; 83:1237–1247. [PubMed: 10877389]

Nation DP, Malmo J, Davis GM, Macmillan KL. Accuracy of bovine pregnancy detection using transrectal ultrasonography at 28 to 35 days after insemination. Aust Vet J. 2003; 81:63–65. [PubMed: 15084014]

NRC. Nutrient Requirements of Dairy Cattle. 7. Natl. Acad. Press; Washington, DC: 2001. rev. ed

Opsomer G, Grohn YT, Hertl J, Coryn M, Deluyker H, de Kruif A. Risk factors for postpartum ovarian dysfunction in high producing dairy cows in Belgium: A field study. Theriogenology. 2000; 53:841–857. [PubMed: 10730974]

Romano JE, Thompson JA, Forrest DW, Westhusin ME, Tomaszweski MA, Kraemer DC. Early pregnancy diagnosis by transrectal ultrasonography in dairy cattle. Theriogenology. 2006; 66:1034–1041. [PubMed: 16584765]

Romano JE, Thompson JA, Kraemer DC, Westhusin ME, Forrest DW, Tomaszweski MA. Early pregnancy diagnosis by palpation per rectum: Influence on embryo/fetal viability in dairy cattle. Theriogenology. 2007; 67:486–493. [PubMed: 17030359]

Rosenbaum, A.; Warnick, LD. Pregnancy diagnosis in dairy cows by palpation or ultrasound: A survey of US veterinarians. Proc. 37th AABP Annual Mtg; Fort Worth, TX. Auburn, AL: Am. Assoc. Bovine Pract. (AABP); 2004. p. 198

Salasel B, Mokhtari A, Taktaz T. Prevalence, risk factors for and impact of subclinical endometritis in repeat breeder dairy cows. Theriogenology. 2010; 74:1271–1278. [PubMed: 20728926]

Santos JE, Cerri RL, Ballou MA, Higginbotham GE, Kirk JH. Effect of timing of first clinical mastitis occurrence on lactational and reproductive performance of Holstein dairy cows. Anim Reprod Sci. 2004a; 80:31–45. [PubMed: 15036513]

Santos JE, Thatcher WW, Chebel RC, Cerri RL, Galvao KN. The effect of embryonic death rates in cattle on the efficacy of estrus synchronization programs. Anim Reprod Sci. 2004b; 82–83:513–535.

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Santos TM, Bicalho RC. Diversity and succession of bacterial communities in the uterine fluid of postpartum metritic, endometritic and healthy dairy cows. PLoS ONE. 2012; 7:e53048. [PubMed: 23300859]

Silke V, Diskin MG, Kenny DA, Boland MP, Dillon P, Mee JF, Sreenan JM. Extent, pattern and factors associated with late embryonic loss in dairy cows. Anim Reprod Sci. 2002; 71:1–12. [PubMed: 11988367]

Silva E, Sterry RA, Kolb D, Mathialagan N, McGrath MF, Ballam JM, Fricke PM. Effect of interval to resynchronization of ovulation on fertility of lactating Holstein cows when using transrectal ultrasonography or a pregnancy-associated glycoprotein enzyme-linked immunosorbent assay to diagnose pregnancy status. J Dairy Sci. 2009; 92:3643–3650. [PubMed: 19620645]

Tenhagen BA, Drillich M, Surholt R, Heuwieser W. Comparison of timed AI after synchronized ovulation to AI at estrus: Reproductive and economic considerations. J Dairy Sci. 2004; 87:85–94. [PubMed: 14765814]

Vasconcelos JLM, Silcox RW, Lacerda JA, Pursley JR, Wiltbank MC. Pregnancy rate, pregnancy loss, and response to heat stress after AI at 2 different times from ovulation in dairy cows. Biol Reprod. 1997; 56(Suppl 1):140.

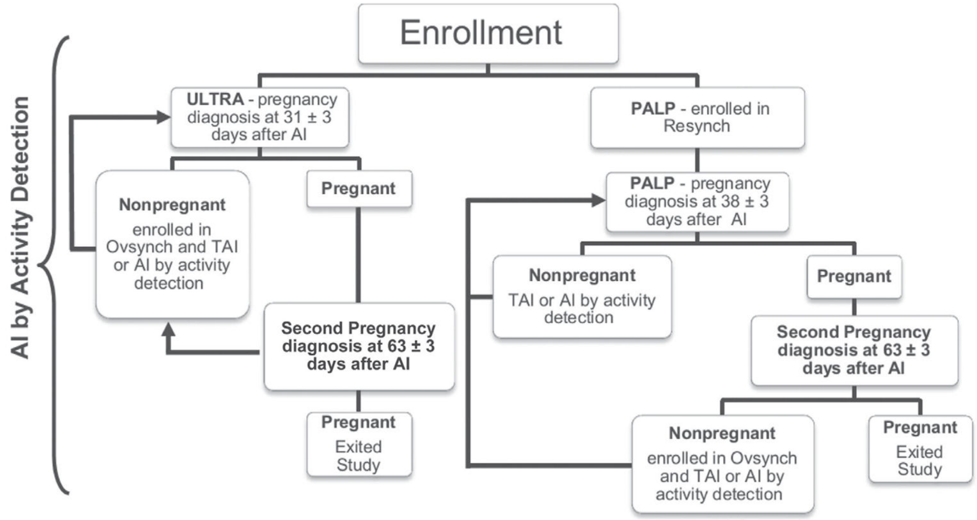

Figure 1.

Flowchart illustrating the timeline for assignment and reproductive management of cows by pregnancy diagnosis group. Cows were enrolled in the study at a first pregnancy diagnosis at 31 ± 3 d after AI (by use of ultrasonography 31 ± 3 d after AI; ULTRA) or 38 ± 3 d after AI (by palpation per rectum 38 ± 3 d after insemination; PALP). Two levels of activity were used as a reference for artificially inseminating cows: an activity level of ≥2 when a cow was coded in DairyComp 305 (Valley Agricultural Software, Tulare, CA) as open (nonpregnant) and an activity level of ≥3 when the pregnancy status of the cow was unknown. TAI = timed AI.

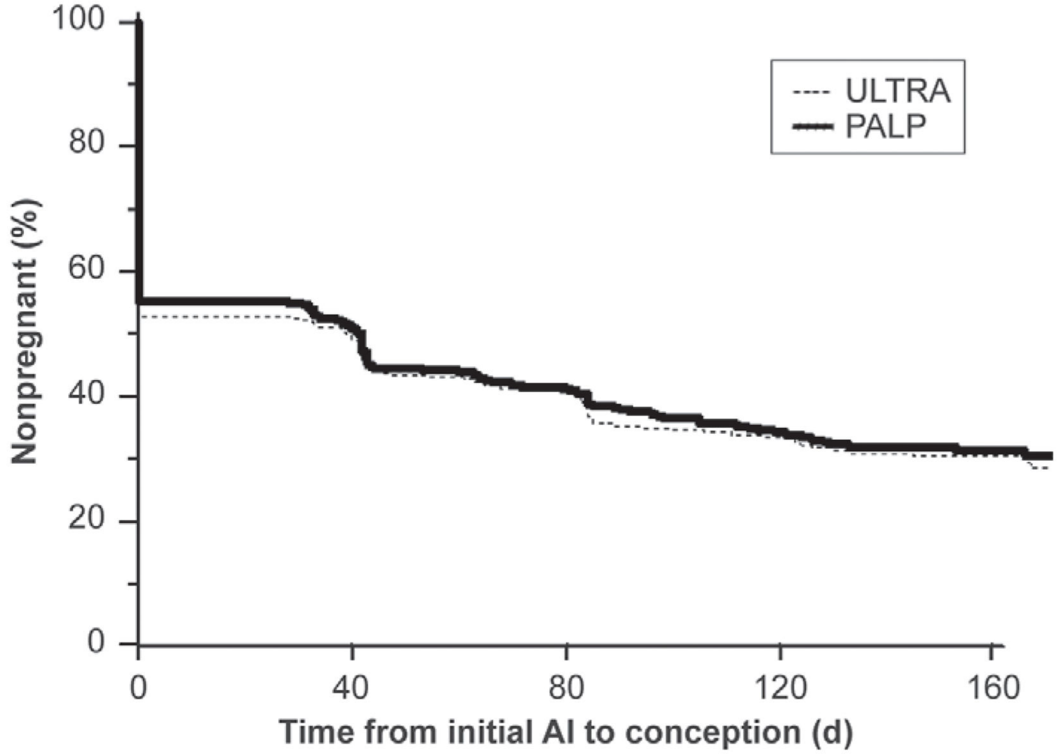

Figure 2.
Kaplan-Meier survival analysis illustrating the effect of pregnancy diagnosis method: use of ultrasonography 31 ± 3 d after AI (ULTRA) versus use of palpation per rectum 38 ± 3 d after AI (PALP) on time to conception (a cow was labeled with a pregnant outcome if diagnosed pregnant at a second pregnancy diagnosis performed at 63 ± 3 d after AI) after initial AI (P = 0.53), where initial AI was the last AI before the cow was enrolled in the study.

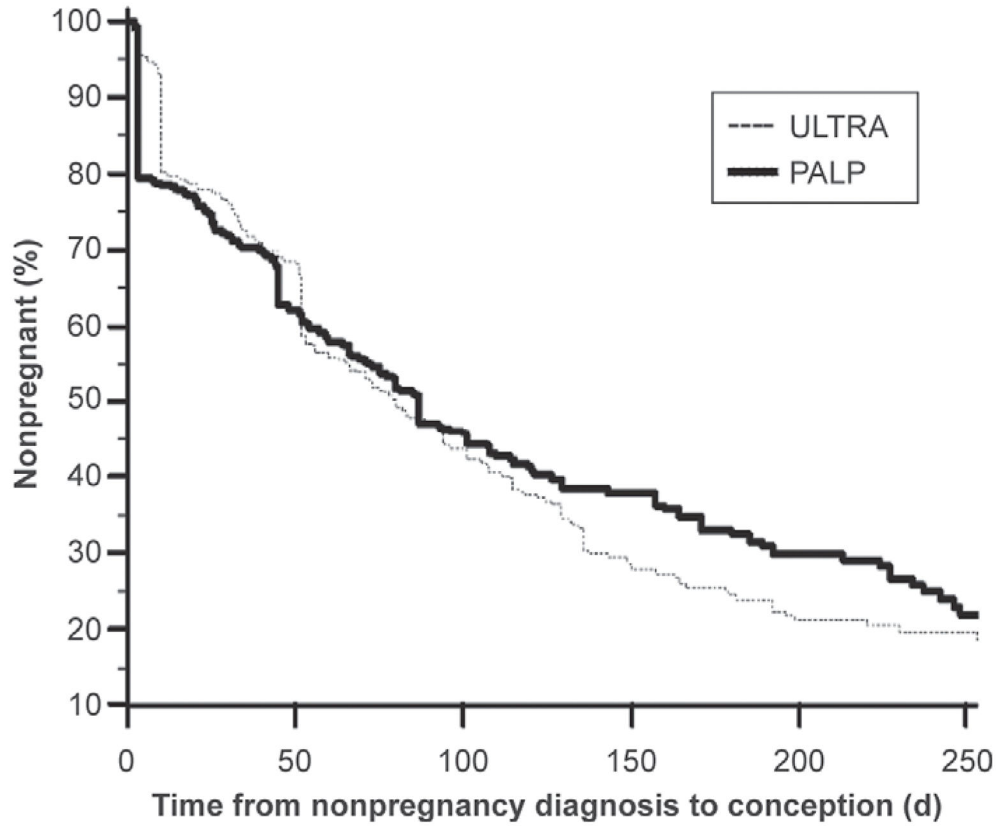

Figure 3.

Kaplan-Meier survival analysis illustrating the effect of pregnancy diagnosis method: use of ultrasonography 31 ± 3 d after AI (ULTRA) versus use of palpation per rectum 38 ± 3 d after AI (PALP) on time to conception (time from a nonpregnant diagnosis from the AI before enrollment to a pregnant outcome diagnosed at a second pregnancy diagnosis, 63 ± 3 d after insemination) for cows diagnosed nonpregnant and re-inseminated after enrollment in the study (P = 0.35).

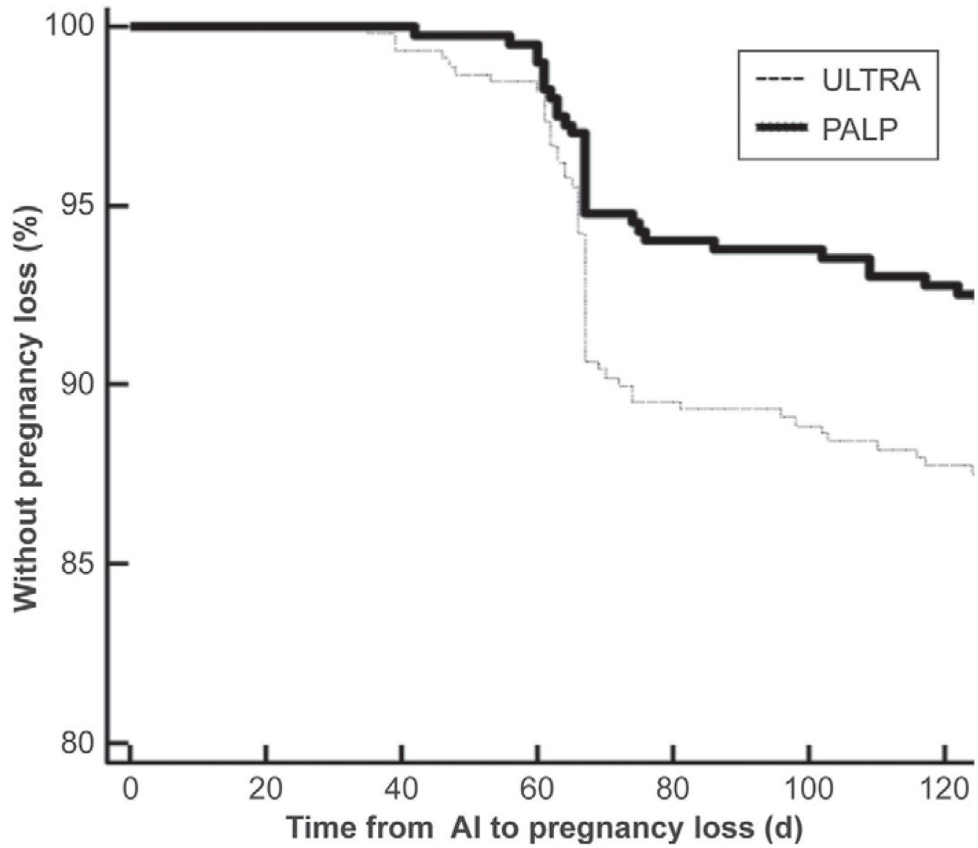

Figure 4.

Kaplan-Meier survival analysis illustrating the effect of pregnancy diagnosis method: use of ultrasonography 31 ± 3 d after AI (ULTRA) versus use of palpation per rectum 38 ± 3 d after AI (PALP) on time to pregnancy loss for pregnancy losses occurring after enrollment in the study ($P = 0.03$). Pregnancy loss was defined as a pregnant diagnosis at 31 ± 3 d after insemination (ULTRA) or at 38 ± 3 d after insemination (PALP) followed by AI by estrus detection using electronic activity monitors or a nonpregnant diagnosis at second pregnancy diagnosis at 63 ± 3 d after insemination.

Table 1

Cox's proportional hazard regression showing the effect of pregnancy diagnosis method, assisted parturition, metritis, retained placenta, and parity on the hazard of conception[1]

Variable	Regression coefficient (SE)	Hazard ratio (95% CI)	*P*-value
Pregnancy diagnosis method[2]			
ULTRA	Referent		0.57
PALP	−0.03 (0.06)	0.97 (0.86; 1.08)	
Assisted parturition			
Yes	Referent		0.04
No	0.24 (0.12)	1.27 (1.00; 1.60)	
Metritis			
Yes	Referent		0.02
No	0.18 (0.08)	1.20 (1.03; 1.40)	
Retained placenta			
Yes	Referent		0.0003
No	0.41 (0.11)	1.51 (1.20; 1.89)	
Parity			
1	0.008 (0.07)	1.01 (0.86; 1.17)	0.36
2	−0.08 (0.08)	0.92 (0.78; 1.07)	
>2	Referent		

[1]A cow was labeled with a pregnant outcome if diagnosed pregnant at a second pregnancy diagnosis performed at 63 ± 3 d after insemination.

[2]ULTRA = pregnancy diagnosis by use of ultrasonography 31 ± 3 d after AI; PALP = pregnancy diagnosis by palpation per rectum 38 ± 3 d after insemination.

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Table 2

Logistic regression model evaluating the effect of pregnancy diagnosis method, parity, type of AI, and their interaction on the odds of pregnancy loss

Variable	PL[1]	Adjusted odds ratio	*P*-value
Main effects			
Pregnancy diagnosis method[2]			
PALP	5.2% (402)	Referent	0.01
ULTRA	9.8% (449)	2.00	
Parity			
1	6.5% (369)	Referent	0.48
2	8.8% (284)	1.42	
>2	8.0% (198)	1.3	
Type of AI[3]			
TAI	6.5% (659)	Referent	0.01
Activity	11.5% (192)	1.96	
Interactions			
PALP × TAI	4.5% (306)	Referent	0.007
PALP × Activity	7.3% (96)	1.64	
ULTRA × TAI	8.2% (353)	1.86	
ULTRA × Activity	15.6% (96)	3.86	

[1]Percent of animals presenting a pregnancy loss, which was defined as a pregnancy diagnosis at 31 ± 3 d after insemination (ULTRA) or at 38 ± 3 d after insemination (PALP) followed by AI by estrus detection using electronic activity monitors or a nonpregnant diagnosis at the second pregnancy diagnosis at 63 ± 3 d after insemination; values in parentheses correspond to the number of cows within each described category.

[2]ULTRA = pregnancy diagnosis by use of ultrasonography 31 ± 3 d after AI; PALP = pregnancy diagnosis by palpation per rectum 38 ± 3 d after insemination.

[3]TAI = timed AI by use of Ovsynch or Resynch; Activity = insemination by estrus detection based on electronic activity monitors.

Table 3

Logistic regression model evaluating the effect of pregnancy diagnosis method, type of AI, and their interaction on the odds of conception at first and second pregnancy diagnoses[1]

Variable	CR at P1	Adjusted odds ratio	*P*-value for P1	CR at P2	Adjusted odds ratio	*P*-value for P2
Main effects						
Pregnancy diagnosis method[2]						
PALP	26.4 (269)	Referent	0.69	20.1 (269)	Referent	0.46
ULTRA	28.6 (290)	1.08		18.3 (290)	0.85	
Type of AI[3]						
TAI	26.4 (511)	Referent	0.06	18.2 (511)	Referent	0.06
Activity	39.6 (48)	1.8		29.2 (48)	1.9	
Interaction						
PALP × Activity	20.0 (15)	Referent	0.05	20.0 (15)	Referent	0.13
PALP × TAI	26.8 (254)	1.46		20.1 (254)	1.01	
ULTRA × Activity	48.5 (33)	3.76		33.3 (33)	2	
ULTRA × TAI	26.1 (257)	1.41		16.3 (257)	0.78	

[1] CR at P1 = conception rate at first pregnancy diagnosis at 31 ± 3 d after insemination (ULTRA) or at 38 ± 3 d after insemination (PALP); CR at P2 = conception rate at second pregnancy diagnosis at 63 ± 3 d after insemination; values in parentheses correspond to the number of cows within each described category.

[2] ULTRA = pregnancy diagnosis by use of ultrasonography 31 ± 3 d after AI; PALP = pregnancy diagnosis by palpation per rectum 38 ± 3 d after insemination.

[3] TAI = timed AI by use of Ovsynch or Resynch; Activity = insemination by estrus detection based on electronic activity monitors.