Comparison of reproductive performance in lactating dairy cows bred by natural service or timed artificial insemination

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ABSTRACT

The objective of this study was to compare reproductive performance of lactating dairy cows bred by natural service (NS) or timed AI (TAI). One thousand fifty-five cows were blocked by parity and enrolled to receive either NS or TAI. Cows in both groups were presynchronized with 2 injections of PGF_{2α} given at 42 and 56 d postpartum. Fourteen days after the last PGF_{2α} injection, cows in the TAI group were enrolled in an Ovsynch protocol (d 0 GnRH; 7 d later, PGF_{2α}; 56 h after PGF_{2α} injection, second dose of GnRH; and 16 h after second GnRH cows were TAI). Cows in the TAI group were resynchronized with an intravaginal insert containing progesterone inserted 18 d after TAI and removed 7 d later when GnRH was given. Cows were examined by ultrasonography on d 32 after TAI; nonpregnant cows received PGF_{2α} and GnRH 56 h later followed by TAI 16 h after the GnRH injection. Nonpregnant cows in TAI group were reinseminated up to 5 times using the same scheme. Cows in the NS group were exposed to bulls 14 d after the second PGF_{2α} injection, and ultrasonography was performed 42 d after exposure to bulls to determine pregnancy status. Nonpregnant cows in the NS group were reexamined by transrectal palpation combined with ultrasound every 28 d until diagnosed pregnant or 223 d postpartum, whichever occurred first. Cows diagnosed pregnant in TAI or NS were reconfirmed 28 d later to determine pregnancy loss. All bulls underwent an evaluation of breeding soundness and were rested for 14 d after 14 d of cow exposure. Health disorders were evaluated up to 70 d postpartum, and body condition score was evaluated at d 70 postpartum. Blood was collected on d 56 and 65 postpartum and analyzed for progesterone to determine cyclicity. The proportion of pregnant cows in the first 21 d of breeding did not differ between groups. The overall 21-d cycle pregnancy rate (PR),

which included a total of 8 and 5 service opportunities for NS and TAI, respectively, was not different between groups (25.7 and 25.0% for NS and TAI, respectively). The daily rate of pregnancy was 15% greater for NS than TAI because cows in NS had a greater PR, which resulted in fewer median days open (111 vs. 116 d). Proportion of pregnant cows at 223 d postpartum was greater in the NS than TAI group (84.2 vs. 74.8%, respectively). Cyclicity did not affect reproductive performance. Cows with body condition score ≥ 2.75 had greater proportion of pregnant cows in the first 21 d of breeding and daily PR in the first 223 d postpartum. Primiparous cows had greater proportion of pregnant cows and daily PR than multiparous cows at 223 d postpartum. In conclusion, the greater proportion of pregnant cows in the NS group was attributed to more opportunities for breeding than in the TAI group.

Key words: timed artificial insemination, natural service, reproductive performance, dairy cow

INTRODUCTION

Despite considerable advantages for AI, a significant number of dairy producers use natural service (NS) for their breeding program. In a survey on bull management practices in California, 84% of the producers reported use of NS as a component of their breeding program (Champagne et al., 2002). The most common use of NS was after unsuccessful AI attempts. In dairy herds located in the northeast region of the United States, reported use of NS as a component of the breeding system varied from 55 to 74% (NAHMS, 2002; Smith et al., 2004). In a study that compared pregnancy rates (PR) between AI and NS in Georgia and Florida dairy herds, the use of NS alone or in combination with AI was reported to be around 70% (de Vries et al., 2005). A survey that examined management practices in 103 herds participating in the Alta Genetics (Watertown, WI) Advantage Progeny Testing Program reported that 43% of herds used a clean-up bull (Caraviello et al., 2006). Nonpregnant cows were moved to the clean-up pen after 6 unsuccessful AI or 232 d postpartum. A

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common perception among dairy producers is that NS is comparable to AI because human errors in estrous detection are avoided with bulls.

Several studies have compared reproductive performance between AI and NS breeding systems. Pregnancy rates obtained from dairy herd records of cows bred by AI or NS were not different (Williamson et al., 1978; Niles et al., 2002), but PR from NS were more variable in NS herds (deVries et al., 2005). Using DHIA herd summary information, actual calving interval was shorter in herds that used NS bulls (Smith et al., 2004). The effects of AI and NS breeding systems on PR by stage of lactation and season over an 8-yr time period showed that the use of NS bulls did not result in meaningful advantages or disadvantages in terms of PR over time (deVries et al., 2005). In contrast to the previously cited reports, a California study that compared calving to conception intervals for cows in AI pens with cows exposed to NS sires found that cows AI had a higher risk for pregnancy across all days in milk cohorts (Overton and Sischo, 2005).

A breeding program that synchronizes ovulation and AI at a fixed time (timed AI; **TAI**) has been developed that allows for 100% of the cows to be submitted to AI, without the need for estrous detection (Pursley et al., 1995). Ample studies have clearly demonstrated an advantage in PR of TAI over insemination at detected estrus (Pursley et al., 1997a, b; Cartmill et al., 2001). However, studies that compare reproductive performance between NS and TAI, 2 breeding systems in which efficiency of estrous detection is not a factor, are lacking. It was hypothesized that the use of TAI would result in a greater 21-d cycle PR than NS because in TAI cows undergo ovulation synchronization, and all eligible cows are inseminated at a fixed time. Therefore, the objective of this study was to compare reproductive performance of lactating dairy cows bred by TAI or NS, 2 breeding system in which estrous detection is not required.

MATERIALS AND METHODS

Animals, Housing, and Diets

The University of Florida Institutional Animal Care and Use Committee approved all procedures involving cows and bulls. The study was conducted between November 2006 and March 2008 in a commercial dairy farm of 2,200 Holstein cows located in north central Florida. Cows were housed in free-stall barns with fans and sprinklers for forced evaporative cooling during the warm season. Four barns (2 TAI; 2 NS), each with a maximum capacity of 180 cows, were used in the study. These barns were similar in design, size, and number of

animals housed and were switched between and within seasons to avoid environmental bias. Resting bulls were housed in bermudagrass pasture lots with portable shades and trees for heat abatement. Lactating cow diets were formulated using the CPM-Dairy cattle ration analyzer (version 3.0.7a, Cornell University, Ithaca, NY; University of Pennsylvania, Kennett Square, PA; and William H. Miner Agricultural Research Institute, Chazy, NY) to meet or exceed the nutrient requirements established by NRC (2001) for lactating Holstein cows weighing 650 kg, consuming 24 kg/d of DM, and producing 45 kg/d of milk containing 3.5% fat and 3.1% true protein during the first 80 d of lactation. The composition of the diets consisted of corn silage, alfalfa hay, ground corn, citrus pulp, cottonseed hulls, expeller soybean meal (SoyPlus, West Central Soy, Ralston, IA), and solvent-extracted soybean meal. Bulls were fed the lactating cow diet during the 2-wk cow exposure period and lactating cow diet weigh back, which averaged 17.2 kg/d of DM per bull, during the 2-wk rest period.

Study Design, Treatments, and Exclusion Criteria

Once a week until completion of the study, lactating Holstein dairy cows were blocked by parity (primiparous and multiparous) and randomly allocated within each block at 42 ± 3 d postpartum into 2 groups, TAI ($n = 543$) and NS ($n = 512$). Before study enrollment, cows underwent a reproductive tract examination and health record evaluation. Cows with uterine infection or adhesion, a displaced abomasum, cesarean section, or fetotomy, and cows that missed any part of their experimental protocol were not included in the study. After enrollment, cows that were sold or died or missed any part of their protocol were removed from the study. Accordingly, 89 cows from the TAI group and 118 cows from NS cows were removed.

TAI Reproductive Management

Cows in the TAI group were presynchronized with 2 injections of $\text{PGF}_{2\alpha}$ (500 μg of cloprostenol sodium, Estroplan; Pfizer Animal Health, New York, NY) given at 42 ± 3 and 56 ± 3 d postpartum. Fourteen days after the second injection of $\text{PGF}_{2\alpha}$ cows were given an injection of GnRH (100 μg of gonadorelin, Fertagyl; Intervet Inc., Millsboro, DE) followed 7 d later by an injection of $\text{PGF}_{2\alpha}$, and a second injection of GnRH 56 h after the last dose of $\text{PGF}_{2\alpha}$. The TAI was performed 16 h after the second injection of GnRH. Eighteen days after TAI, cows received a controlled internal drug-releasing insert (**CIDR**; CIDR Eazi-Breed; Pfizer Animal Health) followed by insert removal and GnRH administration 7 d later on d 25 after TAI. Cows were

Figure 1. Timeline of reproductive events to the first service, blood sample collection, and body score evaluation for cows enrolled at timed AI (TAI) and natural service (NS) breeding programs. BS = blood sample; US = ultrasonography examination; CIDR = controlled internal drug-releasing insert.

evaluated for pregnancy by ultrasonography examination at 32 d after TAI. The reproductive program for TAI cows is illustrated in Figures 1 and 2. The presence of an embryo with a heartbeat was the criterion used to determine pregnancy as described previously (Ginther, 1998). The resynchronization protocol was chosen to maximize reproductive efficiency and to allow cows to be reinseminated immediately after the diagnosis of nonpregnancy. Based on ovarian dynamics at 18 d after AI, a preovulatory follicle is potentially present, and insertion of a CIDR device will maintain this follicle and not allow ovulation in cows with a regressed corpus luteum. When GnRH is administered 7 d later at CIDR insert removal (d 25 after insemination in TAI cows), ovulation of the follicle is expected and a new follicular wave is initiated. Therefore, cows found nonpregnant at 32 d after AI would be ready to receive PGF_{2α} and complete the modified Ovsynch protocol at 35 d from their previous breeding. In this manner, the modified Ovsynch protocol begins 7 d before the diagnosis of nonpregnancy, which shortens the interval between inseminations.

Cows diagnosed pregnant were reexamined by transrectal palpation of the uterus and its contents 28 d later (i.e., 60 d gestation) to reconfirm pregnancy sta-

tus and to identify pregnancy loss. Cows diagnosed not pregnant at 32 d after TAI were administered PGF_{2α}, followed with an injection of GnRH at 56 h after PGF_{2α}, and TAI was performed 16 h after GnRH. Nonpregnant cows were resynchronized again with the same TAI protocol until diagnosed pregnant or at a maximum of 223 d postpartum.

NS Reproductive Management

Cows in the NS group received PGF_{2α} at d 42 ± 3 and 56 ± 3 and were moved to a bull pen at 70 ± 3 d postpartum. Cows were moved to the bull pen 14 d after the last PGF_{2α} (70 ± 3 d postpartum) to improve synchronized estrus and to have the bull breeding at around 80 d postpartum, i.e., similar to the first service in the TAI group. After 42 d of being exposed to the bulls, cows underwent an ultrasonography and transrectal examination to determine pregnancy status, which allowed a diagnosable gestation length in pregnant cows to vary from 28 to 42 d. The reproductive program for NS cows is illustrated at Figures 1 and 2. Gestation age was estimated by measurement of embryo size, presence of a heartbeat by ultrasound (Ginther, 1998), and the diameter of the pregnant uterine horn and length of the

Figure 2. Timeline of reproductive events for nonpregnant cows to the first service and respective services in timed AI (TAI) and natural service (NS) breeding programs. US = ultrasonography examination; CIDR = controlled internal drug-releasing insert.

amniotic vesicle as determined by transrectal palpation (Zemjanis, 1970). Gestation age from 28 to 34 d was determined by ultrasound and from 35 to 56 d by ultrasound in combination with transrectal palpation.

Cows diagnosed nonpregnant were reexamined for pregnancy status by ultrasound 28 d later to detect pregnancy in cows that were less than 28 d of gestation at the previous ultrasound diagnosis (i.e., 28 to 56 d pregnant), utilizing the same criteria described above for ultrasound and transrectal palpation. This procedure was similar for subsequent groups assigned weekly to the NS group up to 223 d postpartum. Cows diagnosed pregnant were reconfirmed 28 d later to identify pregnancy loss. The bull-to-cow ratio in the NS herds was 1 bull per 20 nonpregnant cows. Day postpartum when pregnancy occurred in NS bred cows was calculated by subtracting the day of pregnancy from the days postpartum when pregnancy was diagnosed. For example, a cow diagnosed pregnant 32 d at 130 d postpartum was pregnant at 98 d postpartum (i.e., 130 to 32 d). The interval between services in the TAI group was 35 d because of the process of carrying out the resynchronization protocol. Therefore, days postpartum when pregnancy occurred to first, second, third, fourth, or fifth service for cows in the TAI group were classified as follows: d 80 ± 3 , first service; d 115 ± 3 , second service; d 150 ± 3 , third service; 185 ± 3

d, fourth service, and 220 ± 3 d, fifth service. For cows in the NS group, when pregnancy was diagnosed from 28 to 56 d, the first, second, third, fourth, fifth, sixth, seventh, or eighth services were classified at d 70 to 90, d 91 to 111, d 112 to 132, d 133 to 153, d 154 to 174, d 175 to 195, d 196 to 216, and d 217 to 223 postpartum, respectively. A cow in the NS group diagnosed 40-d pregnant at 150 d postpartum would have conceived at 110 d (i.e., $150 - 40$ d) postpartum or at her second service.

Pregnancy Loss

Because of the reproductive management schemes, NS cows were at different stages of gestation when pregnancy was diagnosed than TAI cows, which were consistently diagnosed pregnant at 32 d. Consequently, stage of gestation when pregnancy was diagnosed in the NS group was categorized by 4 d intervals (28 to 32, 33 to 36, 37 to 40, 41 to 44, 45 to 48, 49 to 52, and 53 to 56) in an attempt to discern the effect of embryonic age on pregnancy loss.

Bull Management

Twenty-six bulls 18 mo old at the beginning of the study were used. All bulls underwent a breeding

soundness evaluation according to the guidelines of the Society for Theriogenology (Chenoweth, 1992) before cow exposure. In addition, bulls were tested for *Tritrichomonas fetus* using a smegma sample cultured in a modified diamond media (InPouch TF, Biomed Diagnostics, White City, OR). Both of these tests were performed every 3 mo for a total of 5 evaluations for each bull. All bulls were rested for 14 d after 14 d of cow exposure. The breeding soundness evaluation included a physical examination, testicular evaluation, measurement of scrotal circumference, and evaluation of sperm morphology and motility following electroejaculation. Only bulls classified as potential satisfactory breeders were used. This classification requires a minimum of 31 cm for scrotal circumference for bulls between 15 to 18 mo of age, a minimum of individual sperm motility of 30%, and 70% normal spermatozoa. In addition, the bulls were tested for bovine viral diarrhea by immunohistochemistry of skin using an ear notch sample. All bulls were vaccinated according to farm operational practices for infectious bovine rhinotracheitis, bovine viral diarrhea, parainfluenza 3, bovine respiratory syncytial virus, leptospirosis, clostridial diseases, and campylobacteriosis.

Milk Production Data and BCS

Milk weights were recorded once a month for 988 cows ($n = 484$ and $n = 504$ for NS and TAI, respectively). First measurements occurred at different days after calving for the first monthly sample depending upon day of parturition and day of the monthly milk test for cow and herd, respectively. Because cows were randomly assigned to the treatments on a weekly basis, the first day postpartum measurement was balanced between groups. Data for the first 3 mo of lactation were obtained from the DHIA (Raleigh, NC). Cows in both the NS and TAI groups underwent a BCS evaluation at 70 ± 3 d postpartum before introduction to the bulls in NS or before receiving the GnRH injection in TAI, using a scale of 1 to 5 according to Ferguson et al. (1994).

Temperature Humidity Index

The temperature ($^{\circ}\text{F}$) and relative humidity (%) data were obtained from the Florida Automated Weather Network (<http://fawn.ifas.ufl.edu/scripts/reportrequest.asp>). The data were collected from January 2007 to March 2008. The weather station is located in Alachua, Florida, approximately 30 miles from the experimental location. Average daily temperature-humidity index (**THI**) was calculated as described by West (1993): $\text{THI} = \text{temperature } (^{\circ}\text{F}) - [0.55 - (0.55 \times$

$\text{relative humidity})] \times (\text{temperature} - 58)$. The THI was the criterion used to determine effect of season (warm or cold) on reproductive performance. The maximum daily THI was categorized as cool when $\text{THI} < 72$, or warm when $\text{THI} \geq 72$. The THI on the day of the first TAI or the first day of exposure to bulls was used in the statistical analysis.

Blood Sampling and Evaluation of Cyclicity

Blood samples (approximately 6 mL) were collected from a subset of 608 cows ($\text{NS} = 302$ and $\text{TAI} = 306$) before the second $\text{PGF}_{2\alpha}$ of the presynchronization program and again 9 d later, which corresponded to 56 ± 3 and 65 ± 3 d postpartum. Sampling was collected by puncture of the median coccygeal vein or artery using evacuated tubes containing K_2EDTA for plasma separation (Becton Dickinson, Franklin Lakes, NJ). Samples were placed immediately on ice and transported to the laboratory within 5 h of collection. Blood tubes were centrifuged at $1,500 \times g$ for 15 min, and plasma was frozen at -25°C until analysis. Analysis of progesterone in plasma was determined using a solid phase radioimmunoassay (Coat-A-Count, Progesterone In vitro Diagnostic Test Kit, Diagnostic Products Corp., Los Angeles, CA). Plasma concentrations of progesterone of known values were used in duplicates in every assay to calculate inter- and intraassay coefficients of variation. The known samples were plasma from an ovariectomized cow (0.7 ng/mL), a low progesterone sample (1.0 ng/mL), and high progesterone sample (5.0 ng/mL). The inter- and intraassay CV were 5.74 and 11.1%, respectively. Cows were reported as cyclic if they had progesterone plasma concentration equal to or greater than 1.0 ng/mL in at least 1 of the 2 samples, or non-cyclic if both samples were below 1.0 ng/mL.

Health Disorder Monitoring Program and Treatments

Farm personnel were trained and farm operational procedures were created by the herd veterinarian (corresponding author) to provide a reliable source for health monitoring and prompt treatment. All cows underwent a postpartum health monitoring program consisting of daily evaluation of rectal temperature and attitude from d 2 to 10 postpartum. Rectal temperature was determined with the use of a digital thermometer (GLA M500HPDT, Agricultural Electronics, San Luis Obispo, CA) between 0700 and 0900 h, after milking. Retained placenta was defined as the presence of fetal membranes 24 h after calving. Cows that either appeared sick (inappetence, depressed, sunken or tented eyes) or had a rectal temperature $\geq 39.4^{\circ}\text{C}$ were examined for vaginal discharge, urine ketones, displacement

of abomasum, and respiratory disorder. The criterion for diagnosis of metritis was the presence of a watery, brown, fetid discharge from the vulva (i.e., noted after palpation per rectum of the uterus) with rectal temperature $\geq 39.4^{\circ}\text{C}$. Urine was evaluated for ketonuria using urine test strips (Ketostix, Bayer Diagnostics, Tarrytown, NY). Clinical ketosis was characterized by inappetence, depressed attitude, and presence of ketonuria according to the degree of color change in the test strips. Cows were examined for clinical mastitis by herd personnel during each milking. A case of mastitis was characterized by the presence of abnormal milk or by signs of inflammation in one or more quarters or by both situations. Respiratory disorder was categorized by the presence of abnormal lung sounds, cough, increased respiratory rate, decreased milk production, and fever. Cows were examined for clinical lameness on a weekly basis as they walked out of the milking parlor to barns. Cows affected with lameness exhibited an arched back posture while standing and walking and an abnormal gait. Lameness was examined on a tilt table for lesions and treatment by a professional hoof trimmer. Morbidity was defined as the occurrence of at least one health disorder in the first 70 d postpartum.

Statistical Analysis

The outcomes for comparison of reproductive performance between NS and TAI were the proportion of cows pregnant in the first 21 d of breeding, 21-d cycle PR, daily rate of pregnancy in the first 223 d postpartum, median days nonpregnant, and proportion of pregnant cows at 223 d postpartum. To evaluate the 21-d cycle PR in both NS and TAI cows, an assumption was made that cows were eligible to be bred every 21 d in both groups, despite the fact that TAI cows had a breeding opportunity only every 35 d. Therefore, a breeding eligibility period was considered every 21 d up to 223 d postpartum for both groups.

The proportions of pregnant cows during the first 21 d of breeding and at the end of 223 d postpartum were analyzed by logistic regression using PROC LOGISTIC (SAS Institute, 2003). The model included the effects of group (NS vs. TAI), parity (primiparous vs. multiparous), BCS, categorized as ≤ 2.75 or > 2.75 , season of breeding (warm vs. cool), morbidity, and interactions of group with parity, BCS, season of breeding, and morbidity. Modeling was performed using backward stepwise selection with the significance level of stay when $\alpha \leq 0.10$. The model fit statistics were performed by comparison of the difference in the deviances by the likelihood-ratio statistic test. Adjusted odd ratio and 95% confidence intervals (CI) were calculated.

The 21-d cycle PR was analyzed using the GLIMMIX procedure for generalized linear mixed models (SAS Institute, 2003). The model included the effects of group, parity, BCS, season of breeding, and morbidity. The rate of pregnancy in the first 223 d postpartum (daily PR) and median days nonpregnant were analyzed using Cox's proportional hazards regression model (PROC PHREG) and the Kaplan Meyer survival curves (PROC LIFETEST; SAS Institute, 2003). The Cox's model included effects of group, parity, BCS categorized, season of breeding, morbidity, and the interactions of group and season of breeding, and group and morbidity. When interactions were nonsignificant ($P > 0.10$), they were dropped from the model. The adjusted hazard ratios (AHR) and the 95% CI were calculated.

To determine the impact of milk yield in the first 3 mo postpartum on reproductive performance of dairy cows, additional analyses were performed with 988 cows that had milk yield data of the 1,055 cows in the study. The models were the same described above, but included milk yield either as a continuous variable or categorized into quartiles within primiparous and multiparous cows.

Cow days at risk was calculated as the cumulative number of days for cows between exposure to bulls and pregnancy or end of study in the NS group, and between first AI and pregnancy or end of study for the TAI group. The proportion of NS and TAI bred cows affected by health disorders were analyzed by chi-square. Pregnancy loss was analyzed by chi-square analysis (SAS Institute, 2003) and was categorized accordingly with the age of the embryo at the time pregnancy was diagnosed.

Differences with $P \leq 0.05$ were considered significant, and those with $0.05 < P < 0.10$ were considered a tendency.

RESULTS

Assessment of Bulls

Six bulls (23.1%; 6/26) were culled during the study: 2 became lame, 1 developed a bad temperament, 1 had a positive test for *T. fetus*, and 2 were classified as unsatisfactory potential breeders at the completion of the breeding soundness evaluation.

Reproductive Performance

The proportion of pregnant cows in the first 21 d of breeding did not differ ($P = 0.17$) between groups (Table 1). The overall 21-d cycle PR, which included 8 and 5 service opportunities for NS and TAI, respective-

Table 1. Pregnancy rates calculated for every 21-d cycle for cows bred by natural service (NS) or timed AI (TAI) throughout the study

Item	Treatment, % (no./no.)	
	NS	TAI
21-d cycle number ¹		
1	34.2 (175/512)	37.4 (203/543)
2	24.3 (82/337)	28.7 (97/338)
3	13.9 (35/251)	0.0 (0/241)
4	26.8 (56/209)	28.9 (63/218)
5	31.1 (47/151)	27.0 (38/141)
6	15.8 (16/101)	0.0 (0/97)
7	11.8 (10/80)	14.4 (14/97)
8	19.4 (13/67)	—
Total ²	25.7 (421/1,641)	25.0 (417/1,669)

¹To evaluate the 21-d cycle pregnancy rate in both NS and TAI cows, an assumption was made that cows were eligible to be bred every 21 d despite the fact that TAI cows had a breeding opportunity only every 35 d.

²Data from the eighth 21-d cycle were not included in the analysis.

ly, was not different between groups (25.7 and 25.0% for NS and TAI, respectively). However, the rate of pregnancy differed ($P = 0.05$) between groups and was 15% greater (AHR = 1.15; 95% CI = 1.00 to 1.31) for NS than TAI (Figure 3). The median and mean days to pregnancy for NS-bred cows were 111 d (95% CI = 105 to 126) and 131.5 ± 2.3 d, respectively, and for TAI

cows were 116 d (95% CI = 115 to 118) and 137.9 ± 2.9 d, respectively. The survival curves did not differ until 150 d postpartum, when they began to separate. To reinforce this observation, an additional survival analysis was performed for the interval to pregnancy up to 150 d postpartum, in which time nonpregnant cows were censored. In the latter scenario, group did not influence ($P = 0.44$) the rate of pregnancy (AHR = 1.06; 95% CI = 0.91 to 1.23). Nevertheless, at 223 d postpartum, which was the endpoint of the study, the proportion of pregnant cows was greater ($P = 0.001$) for NS than for TAI (NS = 84.8% and TAI = 76.4%). Cow days at risk for pregnancy were not different between NS and TAI (30,978 and 29,424 d, respectively).

Parity, BCS, and Milk Production

Parity did not ($P = 0.15$) affect the proportion of cows pregnant in the first 21 d of breeding. However, a greater proportion ($P < 0.001$) of primiparous cows was pregnant at the end of 223 d of breeding than multiparous cows (87.7 vs. 77.8%) because primiparous cows had a 27% greater ($P = 0.002$) daily PR than multiparous cows (AHR = 1.27; 95% CI = 1.09 to 1.48).

At 70 ± 3 d postpartum, the distribution among BCS categories was not different ($P = 0.66$) between TAI

Figure 3. Survival curves for proportion of nonpregnant cows by days postpartum for cows bred by natural service (NS) or timed AI (TAI) in the first 223 d postpartum. Median interval to pregnancy for NS and TAI groups was 111 d (95% confidence interval [CI] = 104 to 125) and 116 d (95% CI = 115 to 117), respectively. The rate of pregnancy in the 223 d postpartum was greater ($P = 0.05$) for NS than TAI (adjusted hazard ratio = 1.15; 95% CI = 1.00 to 1.31).

Table 2. Effect of health disorders on proportion of pregnant cows in the first 21 d of breeding for cows bred by natural service or by timed AI

Type of disease	Disease,% (no./no.)		P-value
	Yes	No	
Metritis ¹	31.5 (28/89)	35.7 (345/966)	0.49
Lameness	25.6 (11/43)	35.8 (362/1,012)	0.19
Retained placenta	28.5 (15/52)	35.7 (358/1,003)	0.37
Respiratory	17.2 (5/29)	35.9 (368/1,026)	<0.05
Mastitis	22.7 (20/88)	36.5 (353/967)	<0.01
Ketosis	15.4 (6/39)	36.1 (367/1,016)	<0.01
Morbidity	26.4 (76/288)	38.7 (297/767)	<0.01

¹Variable not distributed equally between natural service and timed AI.

and NS: BCS ≤ 2.75 were 52.5% for NS and 53.9% for TAI; BCS > 2.75 were 47.5% for NS and 46.1% for TAI. Cows with BCS greater than 2.75 had increased ($P = 0.004$) proportion of pregnant cows in the first 21 d of breeding (39.9 vs. 32.4%; adjusted odds ratio = 1.46; 95% CI = 1.26 to 1.89) and had an increased ($P = 0.02$) daily PR in the first 223 DIM (AHR = 1.18; 95% CI = 1.03 to 1.35). Despite those effects, no effect ($P = 0.33$) of BCS was observed for the overall cumulative proportions of pregnant cows at the end of the study, which were 78.6% for cows with BCS ≤ 2.75 and 82.4% for cows > 2.75 .

When additional analyses were performed with the subset of 988 cows with milk production data, the daily rate of pregnancy tended ($P = 0.08$) to increase with a concurrent increase in milk yield (AHR = 1.01; 95% CI = 1.00 to 1.01). At the end of 223 DIM, the proportion of pregnant cows was not ($P = 0.23$) affected by milk production in the first 3 mo of lactation.

Cyclic Status, Seasonality, and Health Disorders

Progesterone analyses indicated that 83.1% (251/302) of cows in the NS group and 87.2% (267/306) of cows in the TAI group were cyclic (progesterone > 1 ng/mL in at least 1 sample) before exposure to NS or onset of the TAI protocol. The percentages of cyclic cows were not different ($P = 0.17$) between groups, and cyclic status did not affect ($P = 0.53$) the proportions of pregnant cows in the first 21 d of breeding, which were 35.4% and 38.6% for noncyclic and cyclic cows respectively.

Cows receiving their first breeding during the cool season had increased ($P < 0.01$) pregnancy in the first 21 d of breeding (41.2 vs. 27.7%), 21-d cycle PR (27.5 vs. 22.5%), and daily PR (AHR = 1.22; 95% CI = 1.06 to 1.41). It is important to indicate that no interaction between treatment and heat stress was observed for the proportion of cows pregnant in the first 21 d of breeding or PR.

The only health disorder that was not ($P = 0.03$) distributed equally between TAI and NS was metritis (TAI = 6.6% and NS = 10.3%). However, metritis did not affect the proportion of pregnant cows in the first 21 d of breeding (Table 2). On the other hand, the proportion of pregnant cows in the first 21 d of breeding was less in cows with mastitis ($P < 0.01$), respiratory disorder ($P < 0.05$), ketosis ($P < 0.01$), and morbidity ($P < 0.01$). Morbidity influenced ($P = 0.002$) the rate of pregnancy and healthy cows had a 28% greater daily PR than cows affected by at least one health problem (AHR = 1.28; 95% CI = 1.09 to 1.49).

Pregnancy Loss

The overall pregnancy loss considering the gestation period from d 28 to 56 in the NS group and 32 d for TAI was lower ($P = 0.02$) for NS than TAI-bred cows (10.4% and 15.2%, $P < 0.05$; respectively; Table 3). However, pregnancy loss in the NS group for cows diagnosed pregnant between 28 to 32 d of gestation was the same as for TAI cows at 32 d of gestation (14.9% and 15.2%, respectively), as shown in Table 3.

DISCUSSION

Following the recommendations of the Society for Theriogenology (Chenoweth, 1992) for breeding soundness, a culling rate of 23.1% occurred in bulls used in this study. Kastelic et al. (2000) reported that 20 to 40% of bulls from an unselected population might have reduced fertility. Because these bulls were classified as potential satisfactory breeders before cow exposure and were removed from the study and replaced with sound bulls, the potential for using subfertile bulls in the NS group was minimized. Despite the fact that bulls used in this study had repeatedly breeding soundness evaluations (every 3 mo for a total of 5 evaluations), which is more than what is commonly done, our culling

rates were not greater than what is expected for service bulls in a random population (Kastelic et al., 2000). Our approach to bull evaluation and management was chosen to assure that subfertile bulls could be identified rapidly and removed from the herd to avoid a negative effect on reproduction.

The overall 21-d cycle PR in the NS group of 25.7% was substantially greater than previously reported by de Vries et al. (2005) in NS herds in the states of Florida and Georgia where 21-d cycle PR was 14.0%. Our results can be attributed to the stringent bull management program employed (periodic resting and breeding soundness evaluation) and early removal of unsound bulls, which reduced the potential for deviation in bull fertility during cow exposure. Cows in the TAI group had a first 21-d cycle PR of 37.4%, similar to a PR to first TAI of 37% and 37.9%, reported by Pursley et al. (1997a) and Santos et al. (2009), respectively. The greater proportion of pregnant cows observed in the NS group at the end of the study is attributed to differences in breeding dynamics between groups. In the NS group, bulls had the potential for daily detection of estrus and breeding of nonpregnant cows. On the other hand, because of the TAI resynchronization scheme, nonpregnant cows in this group required 35 d to be reinseminated, and thus the number of days to become pregnant increased. However, within this scenario, up to 223 d postpartum cows in the TAI group had only 5 opportunities to be bred compared with a potential 8 times for cows in the NS group. The increased median number of days to pregnancy observed for TAI cows can also be attributed to this difference in breeding opportunities. A greater number of nonpregnant cows in the NS group had earlier opportunities to be bred than TAI cows under the same 21-d cycle PR; consequently the final outcome for median time to pregnancy favored the NS group.

A greater proportion of pregnant cows and daily PR at 223 d postpartum was observed for primiparous than multiparous cows. This finding indicates that primiparous cows are more fertile than multiparous cows, which agrees with the study of Santos et al. (2009) that reported a greater PR to first service in primiparous cows. In the present study, a greater proportion of pregnant cows in the first 21 d of breeding was found for cows with a BCS >2.75 at 70 d postpartum. This is in agreement with the reports by Santos et al. (2009) and Moreira et al. (2000) in which BCS around 70 d postpartum positively influenced PR. Results from the present study support the concept that BCS at 70 d postpartum is of paramount importance for the establishment and maintenance of pregnancy in high-producing dairy cows.

Table 3. Pregnancy losses according to age of gestation at the initial pregnancy diagnosis in cows bred by natural service (NS) or timed artificial insemination (TAI)

Treatment	Age of pregnancy at first diagnosis (d)	Pregnancy loss, % (no./no.)
TAI	32	15.2 (63/415) ^a
NS	28 to 56	10.4 (45/434) ^b
NS 1	28 to 32	14.9 (18/121)
NS 2	33 to 36	11.6 (11/95)
NS 3	37 to 40	14.9 (10/67)
NS 4	41 to 44	5.3 (2/38)
NS 5	45 to 48	5.7 (3/53)
NS 6	49 to 52	0 (0/11)
NS 7	>52	2.0 (1/49)
TAI and NS total	28 to 56	12.7 (108/849)

^{a,b}Means within same column with different superscripts differ ($P < 0.05$).

Cyclicity did not affect the proportion of pregnant cows in the first 21 d of breeding in the current study. In contrast, Santos et al. (2009) reported a greater PR 58 d post AI to first service in cyclic compared with noncyclic cows. However, in our study the percentage of cyclic cows was greater than expected, which diminished sample size for a valid comparison between cyclic and noncyclic cows and likely limited the power to identify an effect of cyclicity in this study. A bull effect inducing noncyclic animals to ovulate also may have occurred in cows bred by NS. These factors may have contributed to our inability to detect a difference in the proportion of pregnant cows in the first 21 d of breeding between cyclic and noncyclic cows.

Cows bred during the warm season had a lower proportion of pregnant cows in the first 21 d of breeding in both NS and TAI bred cows. This agrees with a previous report (de Vries et al., 2005) that showed conception rates of lactating cows located in Florida decrease to 20% during summer months (June through August). Furthermore, in the present study PR continued to be low during fall (September and October) when THI decreased. During periods of heat stress (summer), overall PR dropped for cows bred by either AI or NS, but no difference in PR was found between groups. In contrast, de Vries et al. (2005) reported that during summer, cows bred by NS had greater 21-d cycle PR than cows bred by AI. However, in the de Vries et al. (2005) study, 21-d cycle PR included all cows eligible for AI during summer. The fact that some dairy producers in Florida choose not to breed cows during summer may have biased the estimates reported by de Vries et al. (2005). During hot weather, some reduction of bull fertility may be expected because of lowered semen quality associated with an increase in abnormal heads, abnormal acrosome, proximal droplets, and a corresponding decrease in motility (Ott, 1986). In our

study, bulls were housed and managed using heat stress abatement conditions designed for lactating dairy cows (shade, fans, evaporative cooling), and changes in bull semen quality may not have occurred or were less severe. Therefore, the depression in PR observed during summer in this study with NS and TAI could be attributed to the effect of heat stress on cow fertility.

The study herd employed a postpartum health monitoring program with the aim to treat disorders promptly. Among the health disorders evaluated, metritis did not affect PR to first service. This result supports the finding by Benzaquen et al. (2007) who reported that cows with metritis did not have impaired fertility. Our finding and those of Benzaquen et al. (2007) reinforced the concept that a postpartum health monitoring program, which allows for early diagnosis and treatment of metritis, may mitigate impairment in fertility related to metritis. On the other hand, the proportion of pregnant cows in the first 21 d of breeding was less in cows with mastitis, respiratory disorder, ketosis, and morbidity.

The greater pregnancy loss observed in TAI-bred cows in our study can be attributed to the earlier time of gestation when pregnancy was diagnosed in this group. In a report by Santos et al. (2004), late pregnancy losses were characterized by corpus luteum maintenance to the end of the differentiation stage, at approximately 42 d of gestation, and were more frequent than pregnancy losses after this stage. This pattern of pregnancy loss agrees with our lower pregnancy losses after 40 d of gestation. Therefore, we attribute the greater pregnancy loss observed in TAI compared with NS bred cows to the days of gestation when diagnosis of pregnancy occurred. Indeed, pregnancy loss was the same when compared at comparable stages of pregnancy in the present study.

The final logistic regression model revealed that the variables affecting the proportion of pregnant cows in the first 21 d of breeding were BCS and an interaction between seasonality and morbidity. The effect of BCS on fertility in dairy cows has been well documented previously and possible reasons are clearly described (Domecq et al., 1997; López-Gatius et al., 2002; Santos et al., 2009).

CONCLUSIONS

The TAI breeding program did not result in a greater 21-d cycle PR compared with NS as initially hypothesized. A greater proportion of pregnant cows at the end of the study occurred in NS-bred cows because they had more opportunities for breeding to occur compared with the TAI reproductive management program. When reproductive performance was compared with the same breeding eligibility period of 21-d cycle PR, there was

no difference between NS and TAI. In conclusion, NS and TAI are 2 breeding systems that can be used strategically to minimize problems related to detection of estrus, but the extended interinsemination interval in TAI reduces daily PR because these cows have fewer opportunities for breeding. Whether or not TAI is more economical than NS warrants investigation.

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