A reproductive management program aimed at increasing reinsemination of nonpregnant dairy cows at detected estrus resulted in similar reproductive performance to a program that favored timed artificial insemination

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ABSTRACT

The objective of this experiment was to compare time to pregnancy and proportion of cows not pregnant 210 d after first service for cows managed for second and subsequent artificial insemination (AI) services with a reproductive management program that promoted reinsemination at detected estrus (AIE) or a program that promoted timed AI (TAI). After first service, lactating Holstein cows were blocked by parity and randomly assigned to d 32 Resynch (D32R; $n = 464$) or AIE Resynch (AIER; $n = 512$). To determine the effect of management strategies on time to pregnancy and cows not pregnant by the end of a 210 d at-risk period after first AI service, cows remained in AIER and D32R until pregnancy or herd exit. Cows in D32R received a GnRH treatment 32 ± 3 d after AI (first treatment intervention; FTI). Nonpregnancy diagnosis was conducted 7 d later by transrectal ultrasonography when nonpregnant cows with a corpus luteum (CL) ≥ 15 mm completed the Resynch protocol (PGF_{2α}, 56 h later GnRH, and 16 to 18 h later TAI) and cows without a CL (NoCL cows) were enrolled in a PreG-Ovsynch protocol (GnRH, 7 d later GnRH, 7 d later PGF_{2α}, 56 h later GnRH, and 16 to 18 h TAI) to receive TAI. For the AIER treatment, nonpregnant cows with a CL ≥ 15 mm observed by transrectal ultrasonography 32 ± 3 d after AI (i.e., FTI) received PGF_{2α} to induce estrus. Cows not AIE within 7 d were enrolled in Resynch (GnRH, 7 d later PGF_{2α}, 56 h later GnRH, and 16 to 18 h TAI). Cows in the NoCL group in AIER were enrolled in PreG-Ovsynch. Detection of estrus was performed based on visual observation of behavioral signs of estrus and tail-paint removal. Binomial data were

analyzed with logistic regression and time to event data with Cox's proportional regression. After the FTI, a greater proportion of cows were AIE in AIER than D32R (36.0 vs. 11.9%) and more cows were AIE within 7 d of the FTI for AIER (25.0%) than D32R (4.8%). Overall pregnancy per AI at 68 ± 3 d after AI did not differ (AIER = 35.5% vs. D32R = 34.7%). The hazard of pregnancy up to 210 d after first AI for all cows enrolled (hazard ratio = 1.04, 95% CI 0.90 to 1.19) and for cows that received treatments only (D32R = 308, AIER = 349; hazard ratio = 1.00, 95% CI 0.85 to 1.19) did not differ. We conclude that a program aimed at increasing the proportion of cows reinseminated at detected estrus by treatment with PGF_{2α} at 32 ± 3 d after AI may be an alternative strategy for dairy farms that prefer or need to inseminate more cows at detected estrus rather than by TAI.

Key words: resynchronization, detection of estrus, reproductive performance, dairy cow

INTRODUCTION

Dairy farm profitability depends on the reproductive performance of the lactating cow herd (Britt, 1985; Meadows et al., 2005; Giordano et al., 2013), which is primarily driven by timing of pregnancy and the proportion of cows that become pregnant during lactation. Effective reproductive management strategies include programs for second and subsequent AI services that aim to reduce the interbreeding interval (Fricke et al., 2003; Wijma et al., 2018) and optimize pregnancy per artificial insemination (**P/AI**; Lopes et al., 2013; Giordano et al., 2016) through a combination of reinsemination of cows at detected estrus (**AIE**) and timed artificial insemination (**TAI**). A typical program consists of a period of AIE after a previous AI service followed by implementation of TAI protocols (Pursley et al., 1995) initiated at the time of (Giordano et al., 2015; Wijma et al., 2018) or 7 d before (Chebel et al., 2003; Giordano et

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al., 2012; Wijma et al., 2017) nonpregnancy diagnosis (NPD). Inclusion of a TAI protocol after the period of AIE ensures timely reinsemination of cows not detected in estrus and, consequently, avoids delayed pregnancy.

In recent years, research has been done on development of reproductive management programs for second and subsequent AI services that promote AIE (hereafter AIE-type strategy) after induction of estrus rather than blanket use of TAI for cows not reinseminated at spontaneous estrus (McArt et al., 2010; Bruno et al., 2013; Chebel et al., 2013; Giordano et al., 2015; Mendonça et al., 2019). These strategies could be beneficial for farms that strive to maximize reinsemination of cows upon detected estrus. Programs designed to promote AIE usually include treatment with $\text{PGF}_{2\alpha}$ to promote expression of estrus in all nonpregnant cows (Bruno et al., 2013; Chebel et al., 2013; Mendonça et al., 2019) or only cows with a corpus luteum (CL; McArt et al., 2010; Giordano et al., 2015). In the latter type of program, cows that do not receive $\text{PGF}_{2\alpha}$ may receive a TAI protocol aimed at improving P/AI of cows without a functional CL (Giordano et al., 2015). Differential treatment based on ovarian structures in this case is expected to increase efficiency and reproductive performance by giving $\text{PGF}_{2\alpha}$ only to cows likely to express estrus in response to the treatment and maximize P/AI of cows not expected to respond to $\text{PGF}_{2\alpha}$. This is relevant because at the typical time after AI (i.e., 28 to 42 d after AI) that these programs are likely to be initiated, a majority but not all lactating dairy cows are expected to have the ovarian structures required to express estrus after $\text{PGF}_{2\alpha}$. Indeed, ~15 to 50% of nonpregnant previously inseminated cows do not present a functional CL at 28 to 35 d after AI (McArt et al., 2010; Giordano et al., 2015; Wijma et al., 2018).

Experiments that evaluated the reinsemination dynamics of lactating dairy cows managed with AIE-type programs (Bruno et al., 2013; Chebel et al., 2013; Giordano et al., 2015) suggested that these programs might be effective when $\geq 40\%$ of eligible cows are inseminated at detected estrus after NPD. For instance, Bruno et al. (2013) and Chebel et al. (2013) reported a ~50-percentage point increase in the total proportion of cows AIE and a faster insemination rate for a program that included $\text{PGF}_{2\alpha}$ for all nonpregnant cows at NPD compared with a program that favored TAI. In a recent study, Giordano et al. (2015) compared pregnancy outcomes at the end of the insemination period in late lactation for an AIE-type resynchronization program versus that of a program that favored TAI. The proportion of cows AIE for second and greater AI service increased, but time to pregnancy and proportion of pregnant cows at the end of the at-risk period of 300

DIM did not differ. Collectively, results from these studies demonstrated the feasibility of increasing the total proportion of cows AIE after NPD. Nevertheless, more research is required to evaluate timing of pregnancy and total proportion of pregnant cows during lactation for AIE-type resynchronization programs implemented at NPD because for commercial dairy farms these are more relevant outcomes than proportion of cows AIE.

Thus, we conducted an experiment to evaluate time to pregnancy and the proportion of lactating dairy cows not pregnant 210 d after first AI service for a resynchronization program aimed at promoting AIE rather than TAI after NPD. This program was compared with a resynchronization strategy that favored TAI by submission of all cows nonpregnant and non-reinseminated at detected estrus after a previous AI to the d 32 Resynch (**D32R**) protocol. To maximize the chances that cows in AIER express estrus and increase the P/AI of cows expected to have low fertility in both treatments, nonpregnant cows without a CL ≥ 15 mm at the expected time of the $\text{PGF}_{2\alpha}$ of both treatments received the PreG-Ovsynch protocol (Giordano et al., 2016). We hypothesized that the resynchronization program designed to promote AIE rather than TAI would increase the overall proportion of cows reinseminated at detected estrus while not negatively affecting time to pregnancy or increasing the total proportion of cows not pregnant for up to 210 d after first service.

MATERIALS AND METHODS

All procedures were approved by the Animal Care and Use Committee of Cornell University.

Cows and General Management

Lactating Holstein cows ($n = 976$) from a commercial dairy farm located in Washington County, New York, were enrolled in this experiment from June 2016 to March of 2018. Cows were housed in freestall barns, with concrete flooring, self-locking headgates, and fans and sprinklers in the feedline. Freestall surfaces were covered with mattresses and sand bedding. During the experimental period, the average number of milking cows was 1,227 (range 1,154 to 1,323) with average daily milk yield of 37 kg/d (range 34 to 39) for all milking cows in the herd. Data for cow numbers and milk yield were retrieved from the dairy herd management software (DairyComp305, Valley Ag Software, Tulare, CA) using the MONITOR command. Cows were milked thrice daily at approximately 8-h intervals and were fed a TMR once daily with ad libitum access to feed and water. The diet was formulated to meet

or exceed the nutrient requirements of lactating dairy cows producing 44 kg/d with 4.0% fat and 3.0% true protein as determined by the Cornell Net Carbohydrate and Protein System (Higgs et al., 2015).

Reproductive Management

For first AI service, cows were synchronized with the Presynch-Ovsynch protocol (PGF_{2α}, 14 d later PGF_{2α}, 11 d later GnRH, 7 d later PGF_{2α}, 56 h later GnRH, and 16 to 18 h TAI) as described in Galvão et al. (2007). Cows were eligible for insemination at detected estrus (**AIE**) any time after the second PGF_{2α} treatment of the Presynch portion of the protocol given at 59 ± 3 DIM. Cows not AIE received the Ovsynch portion of the protocol and TAI at 80 ± 3 DIM. During the experiment, 50.6% (494/976) of cows enrolled in the experiment received first service at detected estrus, whereas the remaining 49.4% (482/976) of cows received TAI.

Detection of estrus was performed by one technician (herd manager 90% of the time and relief technicians from an AI company the rest of the time) once daily in the morning based on a combination of visual observation of behavioral signs of estrus and tail-paint removal. Tail paint (Reveal Livestock Marker Spray Paint, Genex, Shawano, WI) was applied once daily. If detected in estrus, cows were immediately inseminated.

Pregnancy status was determined and evaluation of ovarian structures after NPD was performed by transrectal ultrasonography (**TUS**) of the uterus and ovaries by a veterinarian (7 yr of experience conducting TUS for reproductive examinations) using a portable ultrasound machine (Easi-Scan III, BCF Technology Ltd., Livingston, UK). Pregnancy was determined based on the presence of an amount of anechoic uterine fluid and an embryo of size consistent with the expected stage of pregnancy. The presence of a heartbeat in the embryo was also used as confirmation of embryo viability. The presence of ovarian structures (follicles and corpora lutea) was recorded and their size estimated using on-screen background gridlines composed of squares with 10-mm sides.

Experimental Treatments

This experiment was conducted as a randomized complete block design using parity (i.e., primiparous vs. multiparous) as blocking factor. The dairy herd management software (DairyComp305, ValleyAg Software, Tulare, CA) was programmed to assign eligible cows (i.e., were in the milking herd, received at least one AI service, and were not coded as do not breed)

to treatments on a weekly basis. Briefly, cows were blocked by parity (primiparous vs. multiparous) and then randomly allocated to D32R ($n = 464$) or to AIE Resynch (**AIER**; $n = 512$) treatments. As some cows were inseminated more than once, the total number of AI services was 1,536 and 1,648 for the D32R and AIER treatment, respectively. The experimental treatments (D32R and AIER) consisted of reproductive management strategies for second and subsequent AI services in which cows remained until pregnancy, herd removal due to sale or death, or the end of the experimental period at 210 d after first service. The 210-d at-risk period was calculated for individual cows to ensure that all had the same at-risk period. The treatment code assigned at the time of randomization was used by the dairy herd management software to automatically generate lists of cows for hormonal treatments, TAI, and pregnancy testing based on the experimental treatments on specific days of the week. Except for the time of pregnancy testing when knowledge of experimental treatments was necessary, the treatment code of individual cows was not included in action lists to ensure that research and farm personnel were blind to cow treatment assignment. The treatment code remained unchanged until the next calving so that cows remained in the same treatment until the end of the experiment. The period of treatment allocation extended for more than 12 mo so that enrollment and inseminations for the cold and warm season of the year were represented. Cows that calved after an initial pregnancy during the experiment were randomized when they became eligible for the experiment in the subsequent lactation.

Cows from both experimental treatments were eligible to receive AIE any time after AI. The first experimental treatment intervention (**FTI**) was at 32 ± 3 d after a previous AI for both treatments. Thus, cows in the D32R treatment not inseminated at detected estrus by 32 ± 3 d after AI (time of the FTI) received the first GnRH (86 µg of gonadorelin diacetate tetrahydrate; Fertagyl, Merck Animal Health, Summit, NJ) treatment of the D32R protocol (Fricke et al., 2003) irrespective of their pregnancy status. Seven days later, NPD was performed by TUS. Pregnant cows received no further treatment, whereas nonpregnant cows were classified and received further treatments based on ovarian structures (Figure 1). Cows with at least one CL ≥ 15 mm in diameter and at least one ovarian follicle ≥ 10 mm in diameter (hereafter CL cows) completed the D32R protocol by receiving PGF_{2α} immediately after NPD (500 µg of Cloprostenol sodium; Estrumate, Merck Animal Health), a GnRH treatment 56 h later, and insemination 16 to 18 h after GnRH. Cows that did not meet these criteria (hereafter **NoCL** cows) received

the PreG-Ovsynch protocol (Giordano et al., 2016) immediately after NPD (GnRH, 7 d later GnRH, 7 d later PGF_{2α}, 56 h later GnRH, and 16 to 18 h TAI; Figure 1). Thus, CL cows in the D32R treatment received TAI 42 ± 3 d after a previous AI, whereas NoCL cows received TAI 56 ± 3 d after a previous AI. Cows in the AIER treatment not reinseminated after detected estrus by 32 ± 3 d after AI underwent NPD (i.e., FTI). Nonpregnant cows were classified based on ovarian structures as described for cows in the D32R treatment. In AIER, CL cows received a PGF_{2α} treatment immediately after NPD to promote expression of estrus and receive insemination if detected in estrus. Cows not in AIE within 7 d of the PGF_{2α} treatment were enrolled in the Resynch protocol (GnRH, 7 d later PGF_{2α}, 56 h later GnRH, and 16 to 18 h TAI) to receive TAI 49 ± 3 d after AI. Conversely, NoCL cows were immediately enrolled in the PreG-Ovsynch protocol to receive TAI 49 ± 3 d after previous insemination. All inseminations (i.e., AIE and TAI) during the study were conducted using conventional semen from 170 sires. The average number of inseminations per sire was 18 with a range of 1 to 57.

To better replicate the management conditions of commercial dairy farms that implement resynchronization of estrus and ovulation programs that use ovarian

status for decision-making, cows in this experiment remained in the same treatment, and therefore the same reproductive management strategy until the end of the experiment. Cows may have received a different resynchronization of estrus or ovulation protocol (i.e., for CL or NoCL cows for each respective treatment) after each AI depending on ovarian structures present at the time of NPD. After each AI service, cows were eligible for AIE and received the FTI of the D32R or AIER treatment only if not reinseminated at detected estrus by 32 ± 3 d after AI. As a result, some cows never received TAI during the experiment, whereas others received TAI once or multiple times. Moreover, cows in both treatments received the respective synchronization of estrus or ovulation protocol for CL or NoCL cows after each AI depending on ovarian structures present at the time of NPD. Collectively, this approach reflected the actual conditions of dairy farms where cows are enrolled in a program for second and greater AI services until they either become pregnant, are no longer eligible for AI, or leave the herd due to sale or death.

Reconfirmation of pregnancy was performed 68 ± 3 d after AI by TUS in all cows confirmed pregnant at the initial pregnancy examination unless the cow was reinseminated at detected estrus or left the herd. A pregnancy loss outcome was recorded for cows confirmed

Figure 1. After first service, cows were blocked by parity and randomly assigned to 2 management strategies for second and subsequent AI services. Cows enrolled in the d 32 Resynch (D32R; $n = 464$) treatment received GnRH 32 ± 3 d after AI and nonpregnancy diagnosis (NPD) was conducted 7 d later by transrectal ultrasonography (TUS). Nonpregnant cows with a corpus luteum (CL) ≥15 mm (CL cows) completed the Resynch protocol. Cows without a CL present at NPD (NoCL cows) were enrolled in a PreG-Ovsynch protocol to receive timed AI (TAI). For cows enrolled in the reinsemination at detected estrus (AIE) Resynch (AIER; $n = 512$) treatment, nonpregnant cows based on TUS 32 ± 3 d after AI and with a CL ≥15 mm received a PGF_{2α} treatment to induce estrus. Cows not inseminated in estrus within 7 d were enrolled in Resynch. The NoCL cows in AIER were enrolled in PreG-Ovsynch. Cows remained in AIER and D32R until pregnancy, the end of the experiment at 210 d after first service or herd exit. FTI = first treatment intervention for cows in both treatments at 32 ± 3 d after AI.

pregnant at the initial examination and nonpregnant at the time of reconfirmation or if reinseminated at detected estrus after confirmation of pregnancy. Cows not pregnant at the time of re-confirmation received treatment based on ovarian structures present. All cows regardless of experimental group received the same synchronization of ovulation treatments. Cows bearing a CL received PGF_{2α} to promote expression of estrus and be inseminated if detected in estrus. Cows not AIE within 7 d after the PGF_{2α} treatment received TAI after the Ovsynch protocol. Cows not bearing a CL were enrolled in the PreG-Ovsynch protocol to receive TAI.

Statistical Analyses

A sample size calculation was performed in R (R Core Team, Vienna, Austria) to assess the non-inferiority of the AIER treatment compared with the D32R treatment as previously described in Chow et al. (2008). Based on an expected hazard ratio (**HR**) of 1.20 for time to pregnancy with an overall probability of the event occurrence equal to 0.90, a total of 413 cows per treatment were needed for a 1-sided non-inferiority test with a probability of type I error (α) of 5%, and a probability of type II error (β) of 20%.

Time to pregnancy was analyzed using Cox's proportional hazard regression with the PHREG procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC). Treatment and parity were forced in all time-to-event models. Two models were used to evaluate the hazard of pregnancy during the experiment. The first model evaluated time to pregnancy for up to 210 d after the first service for all cows enrolled in the experiment (D32R = 464; AIER = 512), whereas the second model evaluated time to pregnancy only for cows that received the resynchronization of estrus or ovulation protocols of the treatments at least once during the experiment (D32R = 308; AIER = 349). In both cases, a cow was considered pregnant if pregnancy was maintained up to 68 ± 3 d of gestation. Cows that left the herd due to sale or death were right-censored. For time to pregnancy data, the effect of milk yield in 305 d of lactation [low (primiparous $\leq 9,933$ kg, multiparous $\leq 11,371$ kg); medium (primiparous 9,934 to 10,645 kg, multiparous 11,372 to 12,823); and high (primiparous $\geq 10,646$ kg, multiparous $\geq 12,824$)] was offered to the model. The proportional hazard assumption for time to event data analysis was evaluated by graphical examination of the $\log[-\log(\text{survival probability})]$ function obtained from the PROC LIFETEST of SAS. The assumption of proportional hazards was met for all models. Kaplan-Meier survival curves were created to illustrate time to pregnancy using the survival analysis option in Med-

Calc (version 12.5.0.0; MedCalc Software bvba, Ostend, Belgium).

Binary outcomes (i.e., proportion of cows reinseminated before and after the FTI, proportion of cows AIE, P/AI, and proportion of cows not pregnant at the end of the observation period) were analyzed using logistic regression with the GLIMMIX procedure of SAS fitting a binomial distribution. The proportion of cows that were reinseminated after the FTI was compared for each of 7-d periods after the FTI for up to 28 d after the FTI. For the last period (i.e., 22 to 28 d), the proportion of cows reinseminated after the FTI was analyzed using the Fisher's exact test calculated with the FREQ procedure of SAS because in one of the treatments 0% of the cows had a positive outcome. For all logistic regression models, goodness of fit was evaluated using the ratio between the Pearson statistic and its degrees of freedom. In all cases, model fit was deemed acceptable based on a ratio of approximately 1. Experimental treatment (D32R vs. AIER), parity (primiparous vs. multiparous), and the treatment by parity interaction were offered as explanatory variables to all models. In addition, models for proportion of cows AIE and P/AI included the effect of season of insemination (cool vs. warm). The cold season was defined as September 21 to June 20 and the warm season from June 21 to September 20. Models for evaluation of P/AI were also offered sire of insemination as a fixed effect. The final model for each outcome was selected by backward elimination of explanatory variables with $P > 0.10$ and determination of the lowest value for the Akaike information criterion. Treatment group and parity were forced in all models. Season of AI and sire were removed from P/AI models because $P > 0.10$.

All proportions reported are least squares means generated using the LSMEANS statement of PROC GLIMMIX of SAS. However, for simplification of interpretation and for descriptive data, some of the proportions are reported as arithmetic means obtained with PROC FREQ of SAS. All explanatory variables and their interactions were considered significant if $P \leq 0.05$, whereas $P > 0.05$ and ≤ 0.10 was considered a tendency.

RESULTS

Pattern of Reinsemination and Assignment of Cows to Ovarian Status Groups

For all second and subsequent AI services combined, the proportion of cows that received AIE before the FTI for both treatments (i.e., 32 ± 3 d after AI) did not differ [$P = 0.11$; D32R = 58.0% ($n = 1,536$); AIER =

55.2% (n = 1,648); Table 1]. In contrast, the proportion of cows AIE before the FTI was affected by parity [$P < 0.001$; primiparous = 61.8% (n = 1,027); multiparous = 51.2% (n = 2,157)] and season of AI [$P = 0.04$; cold = 58.6% (n = 2,398); warm = 54.5% (n = 786)].

Overall, the total proportion of cows AIE after the FTI among those that received experimental treatments was greater ($P < 0.001$) for the AIER (36.0%; n = 755) than for the D32R (11.9%; n = 658) treatment, but was not affected by parity ($P = 0.57$) or season of AI ($P = 0.28$). The greater number of inseminations at detected estrus after the FTI for the AIER treatment resulted in an increase ($P < 0.001$) in the total proportion of cows AIE (i.e., before and after the FTI) for all second and subsequent services combined [D32R = 62.8% (n = 1,536); AIER = 71.2% (n = 1,648)]. In addition, the total proportion of cows AIE also was affected ($P < 0.001$) by parity [primiparous = 71.4% (n = 1,027); multiparous = 62.6% (n = 2,157)] and season of AI [$P = 0.04$; cold = 69.1% (n = 2,398); warm = 65.1% (n = 786)].

Out of all cows diagnosed nonpregnant during the experiment, 69.6 (n = 639) and 60.6% (n = 771) had a CL present at NPD for D32R and AIER, respectively. The remaining cows were classified as NoCL in both treatments. For CL cows in the AIER treatment, 48.4%

(223/461) received AIE from the time of NPD until the day of the scheduled TAI. Out of those AIE services, 87% (194/223) were conducted after the PGF_{2α} treatment at NPD and before the first GnRH treatment of the Resynch protocol, whereas the remaining 13% (29/223) were conducted after the first GnRH of Resynch and before the morning of the TAI.

P/AI and Pregnancy Loss

Pregnancy per AI at the first pregnancy examination was not compared statistically because it was conducted at different time points but is presented herein for descriptive purposes. Overall, P/AI at 39 ± 3 d after AI for cows in the D32R treatment was 36.3% (233/642) and was not affected ($P = 0.22$) by parity or AI service type (i.e., AIE vs. TAI; $P = 0.90$). In contrast, P/AI for D32R tended ($P = 0.07$) to be greater for CL (39.3%; n = 427) than for NoCL cows (31.5%; n = 180). For the AIER treatment, overall P/AI 32 ± 3 d after AI was 37.9% (279/737) and was not affected ($P = 0.15$) by parity or AI service type ($P = 0.82$) but tended ($P = 0.10$) to be greater for NoCL (43.0%; n = 284) than CL cows (36.9%; n = 453).

Overall, P/AI 68 ± 3 d after AI (i.e., pregnancy reconfirmation conducted at same time after AI for both

Table 1. Pattern of reinsemination before and after the first treatment intervention

Item	Treatment ¹		P-value
	D32R	AIER	
AI before the FTI ²	58.0 $\pm$ 0.01	55.2 $\pm$ 0.01	0.11
LSM $\pm$ SEM	57.2 (878/1,536)	54.2 (893/1,648)	
% (no./total no.)			
AI after the FTI	42.0 $\pm$ 0.01	44.8 $\pm$ 0.01	0.11
LSM $\pm$ SEM	42.8 (658/1,536)	45.8 (755/1,648)	
% (no./total no.)			
From 0 to 7 d	4.8 $\pm$ 0.01	25.0 $\pm$ 0.02	<0.001
	5.3 (35/658)	26.9 (203/755)	
From 8 to 14 d	68.0 $\pm$ 0.02	2.3 $\pm$ 0.01	<0.001
	67.9 (447/658)	2.3 (17/755)	
From 15 to 21 d	0.2 $\pm$ 0.01	71.1 $\pm$ 0.01	<0.001
	0.2 (1/658)	70.9 (535/755)	
From 22 to 28 d ³	—	—	<0.001
	26.6 (175/658)	0.0 (0/755)	

¹Treatment: cows were randomly assigned to the d 32 Resynch (D32R) or reinsemination at detected estrus Resynch (AIER) treatments. Cows in D32R received a GnRH treatment 32 ± 3 d after AI and nonpregnancy diagnosis (NPD) was conducted 39 ± 3 d after AI. Nonpregnant cows with a corpus luteum (CL) ≥ 15 mm (CL cows) completed the Resynch protocol (PGF_{2α}, 56 h later GnRH, 16 to 18 h later TAI). Cows without a CL present at NPD (NoCL cows) were enrolled in a PreG-Ovsynch protocol (GnRH, 7 d later GnRH, 7 d later PGF_{2α}, 56 h later GnRH, and 16 to 18 h later TAI) to receive TAI. For the AIER treatment, nonpregnant cows at 32 ± 3 d after AI and with a CL ≥ 15 mm received a PGF_{2α} treatment to induce estrus. Cows not inseminated within 7 d were enrolled in Resynch (GnRH, 7 d later PGF_{2α}, 56 h later GnRH, and 16 to 18 h later TAI). The NoCL cows in AIER were enrolled in PreG-Ovsynch. Cows remained in AIER and D32R until pregnancy or herd exit.

²FTI = first treatment intervention (32 ± 3 d after AI for both treatments).

³LSM not available because logistic regression could not be conducted due to one of the groups not having any occurrence of the event of interest.

Table 2. Pregnancy per AI (P/AI) for cows that received the experimental treatments

Item	Treatment ¹		P-value
	D32R	AIER	
Overall P/AI 68 ± 3 d after AI			
LSM ± SEM	35.9 ± 0.02	36.9 ± 0.02	0.71
% (no./total no.)	34.7 (223/642)	35.5 (261/735)	
P/AI for AIE services ²			
LSM ± SEM	35.8 ± 0.02	36.9 ± 0.02	0.86
% (no./total no.)	34.7 (26/75)	34.7 (91/262)	
P/AI for TAI services ³			
LSM ± SEM	35.8 ± 0.02	37.1 ± 0.02	0.67
% (no./total no.)	34.7 (197/567)	35.9 (170/473)	

¹Treatment: cows were randomly assigned to the d 32 Resynch (D32R) or reinsemination at detected estrus Resynch (AIER) treatments. Cows in D32R received a GnRH treatment 32 ± 3 d after AI and nonpregnancy diagnosis (NPD) was conducted 39 ± 3 d after AI. Nonpregnant cows with a corpus luteum (CL) ≥15 mm (CL cows) completed the Resynch protocol (PGF_{2α}, 56 h later GnRH, 16 to 18 h later TAI). Cows without a CL present at NPD (NoCL cows) were enrolled in a PreG-Ovsynch protocol (GnRH, 7 d later GnRH, 7 d later PGF_{2α}, 56 h later GnRH, and 16 to 18 h later TAI) to receive TAI. For the AIER treatment, nonpregnant cows at 32 ± 3 d after AI and with a CL ≥15 mm received a PGF_{2α} treatment to induce estrus. Cows not inseminated within 7 d were enrolled in Resynch (GnRH, 7 d later PGF_{2α}, 56 h later GnRH, and 16 to 18 h later TAI). The NoCL cows in AIER were enrolled in PreG-Ovsynch. Cows remained in AIER and D32R until pregnancy or herd exit.

²AIE = cows inseminated based on detection of estrus (visual observation and tail-paint removal).

³TAI = cows inseminated on timed AI.

treatments) for cows AIE before the FTI was not affected ($P = 0.80$) by treatment [D32R = 40.6% ($n = 850$); AIER = 40.0% ($n = 869$)], but tended ($P = 0.07$) to be greater for primiparous (42.7%; $n = 632$) than for multiparous (38.0%; $n = 1,087$) cows. Similarly, overall P/AI 68 ± 3 d after AI among cows that received experimental treatments did not differ ($P = 0.71$; Table 2) for all second and subsequent AI services, for AI services at detected estrus before TAI ($P = 0.86$), and for TAI services after completion of the synchronization of ovulation protocols ($P = 0.67$). In contrast, parity affected ($P = 0.04$) P/AI because it was greater for primiparous (39.4%; $n = 378$) than for multiparous cows (33.5%; $n = 999$).

Pregnancy loss from the time of the first pregnancy examination to the pregnancy reconfirmation at 68 ± 3 d after AI for all AI services combined was not compared statistically because the first examination was conducted at different days after AI. Nevertheless, for informational purposes the percentage of cows with pregnancy loss recorded was 4.3% (10/233) for D32R and 5.8% (16/277) for AIER.

Pregnancy Outcomes

The hazard of pregnancy for up to 210 d after first service was similar ($P = 0.61$; Figure 2A) for the D32R (referent) and AIER treatments (HR = 1.04, 95% CI 0.90 to 1.19), but was affected ($P = 0.001$) by parity because primiparous cows had greater hazard of pregnancy than multiparous cows (HR = 1.29, 95% CI 1.12

to 1.49). Median time to pregnancy was 67 d (95% CI 61 to 75) and 68 d (95% CI 56 to 77) for the D32R and AIER treatments, respectively. Mean time to pregnancy was 88 ± 3 d and 86 ± 3 d for the D32R and AIER treatments, respectively. The hazard of pregnancy was also affected ($P = 0.03$) by milk production because it was lower for cows with high than with low (HR 0.83, 95% CI 0.70 to 0.98) or medium (HR 0.82, 95% CI 0.70 to 0.97) milk yield. The HR for time to pregnancy did not differ for cows with low and medium milk yield (HR 1.01, 95% CI 0.85 to 1.19). At 210 d after first service, the proportion of nonpregnant cows (includes cows sold and dead during study period) tended ($P = 0.06$) to be greater for the D32R (14.5%; $n = 464$) than the AIER treatment (10.7%; $n = 512$), and was greater ($P < 0.001$) for the multiparous (18.1%; $n = 659$) than the primiparous (8.4%; $n = 317$) group. The proportion of nonpregnant cows by 210 d after first service also was affected ($P < 0.001$) by milk production because a greater proportion of cows with low (19.8%; $n = 333$) than medium (9.8%; $n = 322$) and high (9.8%; $n = 321$) milk yield were not pregnant.

When only cows that received the experimental treatments (i.e., at least once during the experiment) were included in the analysis, the hazard of pregnancy from enrollment up to 210 d after first service was similar ($P = 0.96$; Figure 2B) for the D32R and AIER treatments (HR = 1.00, 95% CI 0.85 to 1.19) and was not affected ($P = 0.41$) by milk production. Median and mean days to pregnancy were 91 d (95% CI 84 to 106) and 107 ± 3 d for the D32R treatment and 98 d

(95% CI 88 to 104) and 108 ± 3 d for the AIER treatment. In contrast, parity affected ($P = 0.01$) the hazard of pregnancy because it was greater for primiparous than for multiparous cows (HR = 1.26, 95% CI 1.05

to 1.51). At 210 d after first service, the proportion of nonpregnant cows did not differ ($P = 0.20$) for the D32R (16.4%; $n = 308$) and AIER (13.1%; $n = 349$) treatments and was not affected ($P = 0.11$) by milk production. In contrast, parity affected the proportion of nonpregnant cows by 210 d after first service because it was greater ($P < 0.001$) for multiparous (21.6%; $n = 461$) than primiparous (9.6%; $n = 196$) cows. Cows that received the experimental treatments at least once received them at 116 ± 1.6 and 114 ± 1.3 DIM for D32R and AIER, respectively.

DISCUSSION

In the present experiment, we evaluated the reproductive performance of lactating dairy cows managed with programs for second and greater AI services in which cows received treatments based on ovarian status at key time points after a previous AI. The AIER strategy was designed to maximize reinsemination of cows at detected estrus through resynchronization of estrus with PGF_{2α} if cows had a putative functional CL. The PreG-Ovsynch protocol was used in NoCL cows to maximize their P/AI because these cows were expected to be less likely to display estrus if administered PGF_{2α}. This management strategy, designed primarily to reinseminate more cows at detected estrus as early as possible after a previous AI, was compared with a strategy (i.e., D32R treatment) that relied on resynchronization of ovulation for TAI for all cows rather than provide more opportunities for AIE. In support of our hypothesis, the AIER program increased the overall proportion of cows reinseminated at detected estrus after the time of the FTI for all second and subsequent services combined. In addition, in support of our non-inferiority hypothesis, time to pregnancy was similar between the AIER and D32R treatments when evaluated including all cows enrolled or only cows that received the resynchronization treatments at least once during the experiment. Moreover, the lack of substantial differences in proportion of cows not pregnant at the end of the observation period meant that a similar proportion of cows were expected to leave the herd due to reproductive failure.

Various strategies have been evaluated to maximize the number of cows inseminated upon detection of estrus for second and greater AI services (McArt et al., 2010; Bruno et al., 2013; Chebel et al., 2013; Giordano et al., 2015; Mendonça et al., 2019). However, only a limited number of experiments reported time to pregnancy in cows that remained in the same management strategy from immediately after first service up to the end of the insemination period. This is essential for evaluating the reproductive performance of cows managed with differ-

Figure 2. (A) Kaplan-Meier survival curves for time to pregnancy from enrollment up to 210 d after the first service postpartum for all cows enrolled in the d 32 Resynch (D32R) and reinsemination at detected estrus Resynch (AIER) treatments that failed to conceive after first service. The hazard of pregnancy was not affected ($P = 0.61$) by treatment (hazard ratio = 1.04, 95% CI 0.90 to 1.19). Median time to pregnancy was 67 d (95% CI 61 to 75) and 68 d (95% CI 56 to 77) for the D32R and AIER treatments, respectively. Mean ($\pm$ SE) time to pregnancy was 88 ± 3 d and 86 ± 3 d for the D32R and AIER treatments, respectively. (B) Kaplan-Meier survival curves for time to pregnancy from enrollment up to 210 d after the first service postpartum for cows that received experimental treatments at least once during lactation (D32R = 308, AIER = 349). The hazard of pregnancy was not affected ($P = 0.96$) by treatment (hazard ratio = 1.00, 95% CI 0.85 to 1.19). Median time to pregnancy was 91 d (95% CI 84 to 106) and 98 d (95% CI 88 to 104) for the D32R and AIER treatments, respectively. Mean ($\pm$ SE) time to pregnancy was 107 ± 3 d and 108 ± 3 d for the D32R and AIER treatments, respectively.

ent management strategies for second and subsequent AI services. In agreement with our findings, Giordano et al. (2015) reported that lactating dairy cows managed with a program designed to promote AIE for cows bearing a CL at 32 ± 3 d after AI resulted in similar time to pregnancy as compared with blanket use of the D32R protocol (i.e., cows not treated based on ovarian structures). Despite similar reproductive performance, Giordano et al. (2015) reported increased insemination rate after NPD for the D32R strategy when compared with the AIE-based program. In our experiment, the greater proportion of CL cows AIE than reported by Giordano et al. (2015; 48.4 vs. 38.0%, respectively) likely compensated for the delayed re-insemination of cows that required TAI. In addition, submitting NoCL cows to the PreG-Ovsynch protocol in the D32R treatment in our experiment resulted in TAI at 56 ± 3 d after a previous AI for 27% of the cows. This is in contrast to reinsemination through TAI at 49 ± 3 d after AI for NoCL cows in the AIER treatment. In spite of these differences, results from our study and those of Giordano et al. (2015) suggest that programs aimed at increasing the proportion of cows AIE for second and greater AI may be as effective as other programs that favor blanket use of TAI. Thus, programs that favor AIE may be of interest to farms that either prefer or need to maximize reinsemination at detected estrus rather than through TAI.

The proportion of cows bearing a CL at 32 ± 3 d after AI in the AIER treatment was in the lower end of the expected range for nonpregnant previously inseminated lactating dairy cows (Wijma et al., 2017; Wijma et al., 2018). Nevertheless, our observations are still in agreement with previous studies that evaluated CL presence at this stage after AI (Giordano et al., 2015; Mendonça et al., 2019). Collectively, for the AIER treatment the relatively low proportion of CL cows, and the fact that 52% were not AIE after the PGF_{2α} treatment resulted in only 27% of cows reinseminated before 42 ± 3 d after AI (i.e., 10 d after FTI). In contrast, ~70% of all cows in the D32R treatment (CL cows) were reinseminated within 42 ± 3 d after their previous AI. Thus, the proportion of CL cows that were detected in estrus after NPD in the AIER group was insufficient to offset the benefit of earlier reinsemination of CL cows in the D32R treatment. On the other hand, the greater proportion of cows receiving AI within 42 ± 3 d after AI for the D32R treatment was offset by the reduced reinsemination rate of NoCL cows. Taken together, these data suggest that the similar insemination rate combined with similar overall P/AI for both management strategies contributed to the lack of difference in the rate at which cows became pregnant during lactation.

The effect of the PGF_{2α} treatment on the proportion of cows AIE for second and greater AI services varied widely in previous experiments (McArt et al., 2010; Bruno et al., 2013; Giordano et al., 2015; Mendonça et al., 2019). In the current experiment, 48% of CL cows in the AIER treatment received AIE, which resulted in a 24-percentage-point increase in the total proportion of cows AIE when compared with the D32R treatment. Our results are in agreement with those of McArt et al. (2010) and Giordano et al. (2015) who reported that 49 and 38% of cows with a CL present ~32 d after AI were detected in estrus after a PGF_{2α} treatment. Thus, results from the current and previous experiments evaluating similar AIE-type strategies at a similar range of days after AI (McArt et al., 2010; Giordano et al., 2015) emphasize the need for immediate enrollment of cows not AIE in synchronization of ovulation protocols for TAI. Otherwise, a substantial proportion of cows would have reinsemination delayed, negatively affecting overall reproductive performance.

Our results for the proportion of cows AIE after the PGF_{2α} treatment are in sharp contrast with those of Bruno et al. (2013) and Mendonça et al. (2019) who reported that ~75% of cows were detected in estrus after PGF_{2α}. A key aspect of the latter studies was that cows were housed in open lot housing systems with dirt flooring. Conversely, cows in the current study, McArt et al. (2010) and Giordano et al. (2015) were housed in freestall barns with concrete flooring, which has been associated with reduced expression of estrus in cattle (Britt et al., 1986; Vailes and Britt, 1990; Rodtian et al., 1996). In addition, cows in the experiments of Bruno et al. (2013) and Mendonça et al. (2019) were treated with PGF_{2α} at ~36 d after a previous AI, which may have resulted in more cows having a mature CL responsive to PGF_{2α}. In spite of the fact that in the current experiment no more than 50% of the CL cows were AIE after the PGF_{2α} treatment, the pregnancy outcomes generated for the AIER program were sufficient to achieve non-inferiority for time to pregnancy compared with the D32R program. It is uncertain at the moment whether a greater proportion of cows AIE (like reported by Bruno et al., 2013, and Mendonça et al., 2019) would result in a reduction in time to pregnancy when compared with the pregnancy outcomes generated by D32R program in our experiment. Therefore, additional efforts to increase the proportion of cows AIE after induction of estrus with PGF_{2α} in herds with cows housed under confinement and concrete flooring are warranted.

The choice of reproductive management strategy for reinsemination of lactating cows in commercial dairy farms depends on many factors, primarily the need to

reduce the interval between inseminations and maximize P/AI, the preference to submit cows for AI based on AIE or TAI, as well as resources available and potential constraints of program implementation. Given the lack of differences in pregnancy outcomes for the strategies compared in this and similar experiments (Giordano et al., 2015), the most relevant implications for commercial dairy farms relate to program implementation rather than potential differences in reproductive performance. From a practical perspective, the greatest benefit of AIE-type programs similar to the one evaluated in this experiment would be reinsemination of a greater proportion of cows at detected estrus for second and greater AI services, and a potential reduction of unnecessary GnRH treatments before NPD for pregnant cows (i.e., if NPD for a D32R-like program is conducted at 39 ± 3 d after AI). Collectively, the number of and reliance on hormonal treatments for resynchronization would be reduced. This may be relevant for farms with labor and technological constraints for implementation of reproductive management tasks required for application of synchronization of ovulation protocols or for farms with a preference to reduce their reliance on hormonal treatments. If that is the case and an AIE-type program is the choice, success of such programs depends on implementation of an effective detection of estrus program to maximize the proportion of cows AIE after the PGF_{2α} and P/AI of AIE services.

CONCLUSIONS

In the present experiment, we demonstrated that a reproductive management strategy for management of second and subsequent AI services designed to increase inseminations at detected estrus resulted in more cows reinseminated upon detection of estrus while achieving non-inferiority for time to pregnancy when compared with a strategy that favored TAI services. The results of our study support the notion that dairy farms with effective detection of estrus programs could implement a reproductive management program that attempts to increase reinsemination of cows at detected estrus rather than by TAI.

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