Reproductive performance of replacement dairy heifers submitted to first service with programs that favor insemination at detected estrus, timed artificial insemination, or a combination of both

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ABSTRACT

Our objective was to compare the insemination dynamics and time to pregnancy for up to 100 d after the beginning of the artificial insemination period (AIP) for heifers managed with first artificial insemination (AI) service programs that relied primarily on insemination at detected estrus (AIE) after PGF_{2α} treatments, timed artificial insemination (TAI), or a combination of both. Holstein heifers were randomly assigned to receive first AI service with sex-selected semen after 368 ± 10 d of age with (1) AIE after synchronization of estrus with up to 3 PGF_{2α} treatments every 14 d starting on the first day of the AIP (PGF+AIE; n = 317). Heifers not AIE up to 9 d after the third PGF_{2α} received a 5-d Cosynch protocol with progesterone supplementation [GnRH + controlled internal drug release insert (CIDR)–5 d–CIDR removal and PGF_{2α}–3 d–GnRH and TAI] before TAI. Heifers detected in estrus from CIDR removal and PGF_{2α} until the day before TAI received AIE with no GnRH treatment; (2) 2 PGF_{2α} treatments 14 d apart with the second treatment at the beginning of the AIP (PGF+TAI; n = 334). Heifers received AIE for up to 9 d after the second PGF_{2α} treatment. Heifers not AIE received TAI after the 5-d Cosynch protocol and (3) TAI after the 5-d Cosynch protocol (ALL-TAI; n = 315). Heifers failing to conceive to a previous AI received a subsequent AI with conventional semen at detected estrus or TAI after the 5-d Cosynch protocol. Binomial outcomes were analyzed by logistic regression, whereas time to AI and pregnancy were analyzed with Cox's regression. The hazard of first AI up to 45 d of the AIP was greater for ALL-TAI than for PGF+AIE

[hazard ratio (HR) = 1.72; 95% confidence interval (CI) = 1.45 to 2.03] and PGF+TAI (HR = 1.51; 95% CI = 1.28 to 1.77), but similar for PGF+AIE and PGF+TAI (HR = 1.14; 95% CI = 0.97 to 1.33). A greater proportion of heifers received AIE in PGF+AIE (98.7%) than in PGF+TAI (78.5%). Overall, first service pregnancy per AI did not differ (PGF+AIE = 42.0%; PGF+TAI = 47.3%, ALL-TAI = 43.8%). Time to pregnancy was reduced for ALL-TAI compared with PGF+AIE (HR = 1.20, 95% CI = 1.02 to 1.42), but was similar to that of PGF+TAI (HR = 1.13, 95% CI = 0.96 to 1.33). Time to pregnancy did not differ for PGF+AIE and PGF+TAI (HR = 1.07, 95% CI = 0.91 to 1.25). Median days to pregnancy were 27, 23, and 21 for heifers in PGF+AIE, PGF+TAI, and ALL-TAI, respectively. We concluded that an ALL-TAI program for first service reduced time to pregnancy, albeit a relatively small reduction, when compared with a program that relied primarily on AIE after induction of estrus with PGF_{2α} treatments. The program that combined synchronization of estrus and TAI (PGF+TAI) resulted in similar time to pregnancy than the predominant TAI and pre-dominant AIE programs.

Key words: insemination at detected estrus, 5-d Cosynch, timed artificial insemination, dairy heifers

INTRODUCTION

Reproductive efficiency of dairy heifers is critical for the economics of the heifer enterprise because reducing time to pregnancy reduces the rearing and opportunity costs of replacements (Ettema and Santos, 2004; Lopes et al., 2013; Silva et al., 2015). Traditionally, reproductive management programs for dairy heifers have been based primarily on inseminations after detected estrus (AIE) rather than through synchronization of ovulation for timed AI (TAI; NAHMS, 2018). High estrus detection efficiency and pregnancies per AI (P/AI) for programs that rely primarily on AIE (Stevenson et al.,

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2008; Lopes et al., 2013; Colazo and Mapletoft, 2017) and poor P/AI for TAI services with 7-d Ovsynch-like protocols (Schmitt et al., 1996; Pursley et al., 1997) are likely main reasons for the low adoption of TAI in heifers.

Although estrus expression is usually not a major limitation for the success of AIE-based programs for heifers (Stevenson et al., 2008; Lopes et al., 2013; Colazo and Mapletoft, 2017), total reliance on AIE may lead to suboptimal reproductive performance of the heifer herd because of biological variation in timing of estrus expression and poor estrus detection efficiency due to management constraints. Conversely, the implementation of TAI can improve AI submission rates, reducing the number of days to first service and the interval between subsequent services. In addition, 5-d Cosynch protocols with progesterone supplementation are now an alternative to obtain comparable P/AI than for AI services after spontaneous or PGF_{2α}-induced estrus (Silva et al., 2015; Colazo and Mapletoft, 2017). Thus, TAI programs including 5-d Cosynch for synchronization of ovulation may be an alternative to reduce time to pregnancy by minimizing days to first service and achieving the same or greater P/AI than with programs based on only AIE.

Reproductive management programs for first service that combine inseminations after PGF_{2α}-induced estrus with Presynch-like protocols (i.e., 2 PGF_{2α} treatments 14 d apart) and submission to a TAI protocol 10 to 14 d after Presynch are a compromise between all-AIE and all-TAI programs. These programs can help reduce time to pregnancy after the end of the voluntary waiting period through rapid insemination of most cows after Presynch and immediate submission to TAI of those cows not detected in estrus. Programs such as Presynch-Ovsynch (Chebel et al., 2006; Fricke et al., 2014; Giordano et al., 2016) and Presynch-Cosynch (Stevenson, 2011; Ribeiro et al., 2012) have been extensively evaluated and are widely used for first service management of lactating dairy cows (Caraviello et al., 2006; Ferguson and Skidmore, 2013). In spite of the fact that these programs may effectively improve reproductive performance of dairy heifers, they have not been evaluated extensively in research and are rarely used for heifers in commercial farms (NAHMS, 2018). Indeed, the combination of high estrus detection efficiency after Presynch, good P/AI for AIE services (Stevenson et al., 2000; Colazo and Mapletoft, 2017), and immediate submission to TAI of heifers not detected in estrus after Presynch may reduce time to pregnancy as effectively as all-TAI programs.

Few experiments have evaluated the effect of management strategies that result in different insemination

dynamics on time to pregnancy of dairy heifers. For instance, Lopes et al. (2013) reported reduced days to pregnancy for heifers submitted to first service after induction of estrus with PGF_{2α} every 11 d compared with submission to TAI after a 5- or 7-d Cosynch protocol. Greater P/AI and earlier submission to first service due to fewer days until heifer were eligible for AI favored the AIE-based program. Conversely, more recently, Silva et al. (2015) reported reduced time to pregnancy for an all-TAI than for an all-AIE program. An equal number of days until heifers were eligible for AI and P/AI for TAI than AIE services favored the TAI program, as heifers were inseminated earlier. Therefore, it remains unclear if all-TAI programs including 5-d Cosynch for synchronization of ovulation for first service reduce time to pregnancy when compared with all-AIE programs for dairy heifers. Moreover, the insemination and pregnancy dynamics of heifer managed with protocols similar to Presynch-Ovsynch or Presynch-Cosynch have not been extensively explored.

Thus, our objective was to compare the insemination dynamics and time to pregnancy during the artificial insemination period (**AIP**) for heifers managed with first AI service programs that relied primarily on AIE, TAI, or a combination of both. We hypothesized that systematic use of reproductive management programs for dairy heifers that hasten insemination through all-TAI or programs that combine an intensive period of AIE followed by submission to TAI would reduce time to insemination and time to pregnancy when compared with a program that relied primarily on insemination after PGF_{2α}-induced estrus.

MATERIALS AND METHODS

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

Farms and Heifer Management

Nulliparous Holstein heifers from 2 commercial dairy farms located in New York State were enrolled in this experiment (farm A located in Washington county and farm B located in Wayne county), from November 2015 to February 2017. In both farms, heifers were housed in freestall barns with concrete flooring and self-locking headgates in the feeding lane. Freestall surfaces were covered with mattresses and either sand (farm A) or dried manure bedding (farm B). Heifers were fed a TMR once a day with unlimited access to feed and water. The diet was formulated to meet or exceed the requirements for a Holstein heifer weighing 450 kg and

gaining 0.8 kg/d (Cornell Net Carbohydrate and Protein System; Higgs et al., 2015).

Experimental Treatments

Once a month, heifers >310 d of age ($n = 966$) were stratified by age in days and then randomly allocated to 1 of 3 different treatments by the dairy herd management software (DairyComp305, ValleyAg Software, Tulare, CA). Each individual treatment consisted of a different reproductive management strategy for first AI service with sex-selected semen. Heifers became eligible for insemination (i.e., beginning of the AIP) at 368 ± 10 d of age. Body weight data at 368 ± 10 d of age was available for a subgroup of heifers at each farm. On farm A ($n = 27$), BW was 394 ± 34 (mean $\pm$ SD) kg, whereas on farm B ($n = 105$) it was 383 ± 34 kg.

On farm A, estrus detection for all AI services was performed once a day by a combination of visual observation of behavioral signs of estrus and tail-paint removal, whereas only visual observation was used on farm B. On farm A, heifers were inseminated immediately after detection of estrus, whereas on farm B heifers were inseminated 6 to 12 h after observation of behavioral estrus (performed inseminations twice a day).

Heifers in the **PGF+AIE** ($n = 317$) treatment received AI at detected estrus (AIE) after synchronization of estrus with up to 3 PGF_{2 α} treatments (25 mg of dinoprost tromethamine given s.c., Lutalyse HighCon, Zoetis, New York, NY) every 14 d starting on the first day of the AIP (Figure 1). Heifers not inseminated at detected estrus for up to 9 d after the third PGF_{2 α} treatment received a 5-d Cosynch protocol for synchronization of ovulation and TAI. Thus, TAI for heifers not AIE occurred 45 d after the beginning of the AIP. The 5-d Cosynch protocol consisted of an initial GnRH treatment i.m. (100 μ g of gonadorelin chloride, Zoetis) plus insertion of a controlled internal drug release insert (**CIDR**; 1.38 g of progesterone, Zoetis); 5 d later, 25 mg of PGF_{2 α} s.c. and CIDR removal; and finally a GnRH treatment (100 μ g of gonadorelin) and insemination 3 d after PGF_{2 α} and CIDR removal. The use of TAI in the PGF+AIE group was a last resource to ensure insemination of all heifers within a reasonable amount of time after the beginning of the AIP rather than a preferred method of submission for AI in this group. For the **PGF+TAI** ($n = 334$) treatment, estrus was synchronized with 2 PGF_{2 α} treatments 14 d apart, with the second treatment given at the beginning of the AIP (Figure 1). Heifers not AIE within 9 d of the second PGF_{2 α} treatment were enrolled in the 5-d Cosynch

Figure 1. Graphical depiction of experimental procedures. Holstein heifers from 2 commercial farms were randomly assigned to 1 of 3 treatments before first AI service, namely (1) PGF+AIE ($n = 317$): insemination at detected estrus (AIE) after synchronization of estrus with up to 3 PGF_{2 α} treatments every 14 d starting on the first day of the artificial insemination period (AIP). Heifers not AIE up to 9 d after the third PGF_{2 α} treatment received a 5-d Cosynch protocol with progesterone supplementation (5-d Cosynch = GnRH + controlled internal drug release (CIDR)–5 d–CIDR removal + PGF_{2 α} –3 d–GnRH + timed artificial insemination (TAI)] for synchronization of ovulation before TAI, (2) PGF+TAI ($n = 334$): 2 PGF_{2 α} treatments 14 d apart with the second treatment at the beginning of the AIP. Heifers were AIE for up to 9 d after the second PGF_{2 α} treatment. Heifers not AIE received TAI after the 5-d Cosynch protocol, and (3) ALL-TAI ($n = 315$): TAI after the 5-d Cosynch protocol. In a subgroup of heifers, a blood sample was collected 14 d before the AIP to determine circulating concentrations of progesterone (P4). BC = blood collection.

protocol to receive TAI. Thus, all heifers received first service within 17 d of the beginning of the AIP. Heifers in the **ALL-TAI** ($n = 315$) treatment were submitted to the 5-d Cosynch protocol for synchronization of ovulation and TAI. The beginning of the AIP for this treatment group coincided with the time of PGF_{2 α} treatment and CIDR removal (Figure 1). All heifers in this treatment group received first service within 3 d of the beginning of the AIP.

Heifers from the 3 treatment groups detected in estrus after CIDR removal and the PGF_{2 α} treatment of 5-d Cosynch until the day before TAI received AIE with no GnRH treatment. These inseminations were considered a separate type of AI service for further analyses (i.e., during the synchronization protocol).

Heifers in the 3 treatment groups failing to conceive at first or subsequent inseminations received AI with conventional semen after detection of estrus or TAI. Reinseminations at detected estrus occurred any time after a previous insemination. Heifers diagnosed non-pregnant through transrectal ultrasonography at 31 ± 3 d after a previous insemination were enrolled in the 5-d Cosynch protocol for resynchronization of ovulation and TAI 2 d after nonpregnancy diagnosis. Similar to first service, heifers from the 3 treatment groups detected in estrus after CIDR removal and the PGF_{2 α} treatment of 5-d Cosynch until the day before TAI received AIE with no GnRH treatment. These inseminations were considered a separate type of AI service for further analyses (i.e., during the synchronization protocol).

Pregnancy Diagnosis

In both farms, pregnancy diagnosis was conducted by transrectal ultrasonography (Easi-Scan, BCF Technology Ltd., Livingston, UK) of the reproductive tract and ovaries 31 ± 3 d after AI by a veterinarian on farm A and a technician from the research team on farm B. Heifers reinseminated before pregnancy diagnosis were considered not pregnant to the previous AI.

Blood Sample Collection

In a subgroup of heifers from both farms ($n = 162$), 2 blood samples were collected 14 d apart to determine circulating concentrations of progesterone (**P4**). Samples were collected 14 d before and at the beginning of the AIP (Figure 1). Blood samples (~ 8 to 9 mL) were collected by puncture of the caudal vein or artery using evacuated tubes containing sodium heparin (Vacutainer BD, Franklin Lakes, NJ). After collection, samples were

placed in crushed ice and transported to the laboratory for further processing. Samples were centrifuged at $2,000 \times g$ for 20 min at 4°C. Plasma aliquots were harvested and transferred to Eppendorf vials for storage at -20°C until assayed.

Determination of Progesterone Concentrations

A commercial solid-phase, no-extraction RIA (ImmuChem Coated Tube, MP Biomedicals, Costa Mesa, CA), previously validated for use in bovine (Garbarino et al., 2004; Skenandore et al., 2017), was used to determine plasma concentrations of P4. To assess precision of the assay, control samples with high (6.2 ng/mL) and low (0.3 ng/mL) concentrations of P4 were included at the beginning and end of each assay ($n = 2$ assays). Average detection limit of the assay was 0.1 ng/mL. Average intra-assay coefficient of variation for the high-concentration sample was 9.2%, whereas the interassay coefficient of variation was 15.0%. For the low-concentration sample the average intra-assay coefficient of variation was 29.2%, whereas the interassay coefficient of variation was 27.0%. Heifers were considered cyclic if at least 1 of the 2 samples collected had circulating P4 ≥ 1 ng/mL.

Statistical Analysis

A sample size calculation was performed using the sample size calculation option of WinPepi version 11.51 (Abramson, 2011). According to the calculations performed, 332 heifers per treatment were needed to detect a hazard ratio of 1.25 for time to pregnancy with an average probability of survival at the end of the observation period of 100 d of 5%, a probability of type I error (α) of 5%, and a probability of type II error (β) of 20%. The smaller number of heifers with data available for analysis for some of the treatments (i.e., as compared with the number of heifers required based on the sample size calculation) was due to losses beyond the control of the researchers after initial enrollment.

Time to first service and time to pregnancy after the beginning of the AIP were analyzed using Cox's proportional hazard regression with the PHREG procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC). Treatment was included as a fixed effect and farm as a random effect. For the analysis of time to pregnancy, a heifer was considered pregnant if pregnancy was maintained until at least 66 ± 3 d of gestation. Heifers that left the herd due to death or sale were right-censored. Kaplan-Meier survival curves were created to illustrate time to event outcomes using the survival analysis op-

Table 1. Cyclicity status before the AI period for heifers enrolled in the experiment

Item	Treatment, ¹ % (n)			P-value
	PGF+AIE	PGF+TAI	ALL-TAI	
P4 ² ≥ 1 ng/mL at baseline ³	64.3 (56)	73.1 (52)	69.2 (52)	0.62
P4 ≥ 1 ng/mL at beginning of AIP	67.3 ^a (55)	88.7 ^b (53)	96.2 ^b (53)	<0.01
Cyclic ⁴ at beginning of AIP	92.9 (56)	94.3 (53)	100 (53)	0.20

^{a,b}Different superscripts within a row indicate significant differences ($P \leq 0.05$).

¹Treatment: at 368 ± 10 d of age, heifers received first AI after (1) PGF+AIE: insemination at detected estrus (AIE) after PGF_{2α} treatments 14 d apart (up to 3) starting at the beginning of the AIP. Heifers not AIE within 9 d of the third PGF_{2α} treatment were enrolled in a 5-d Cosynch protocol [5-d Cosynch = controlled internal drug release insert (CIDR) + GnRH-5 d-CIDR-out + PGF_{2α}-3 d-GnRH + TAI] followed by (2) PGF+TAI: AIE after the second of 2 PGF_{2α} treatments 14 d apart. Heifers not AIE within 9 d after the second PGF_{2α} were enrolled in 5-d Cosynch and (3) ALL-TAI: timed AI after a 5-d Cosynch protocol. In a subgroup of heifers from all treatment groups ($n = 162$), 2 blood samples were collected 14 d apart to determine circulating concentrations of progesterone.

²P4 = progesterone.

³Sample collected 14 d before the artificial insemination period (AIP).

⁴P4 ≥ 1 ng/mL in at least one of the two samples collected.

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

Binary outcomes (proportion of heifers with P4 ≥ 1 ng/mL, proportion of heifers AIE vs. TAI, P/AI, and proportion of pregnant heifers at the end of the observation period) were analyzed using logistic regression with the GLIMMIX procedure of SAS. Treatment was included in models as a fixed effect and farm as a random effect. When appropriate (i.e., statistically significant differences were detected among treatments), the least significant difference post hoc mean separation test was used to determine differences between least squares means. The proportion of cyclic heifers at the beginning of the AIP was evaluated with the Fisher's exact test using the FREQ procedure of SAS because in one of the treatments 100% of the heifers had a positive outcome. Pregnancy per AI at 31 ± 3 d after AI was evaluated for AI services at detected estrus, TAI services, and both types of AI services combined. For AI services outcomes, the effect of season of insemination (cold vs. warm) was offered to the models. The cold season was defined as the period from September 21 to June 20 and the warm season from June 21 to September 20.

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. Variables were considered significant if $P \leq 0.05$, whereas P -values >0.05 and ≤ 0.10 were considered a tendency.

RESULTS

Cyclicity Status and Reproductive Outcomes for First AI Service

Among heifers with P4 data available ($n = 162$), 69% had P4 ≥ 1 ng/mL at baseline (14 d before the beginning of the AIP) and the proportion with P4 ≥ 1 ng/mL did not differ ($P = 0.62$) among treatments (Table 1). At the beginning of the AIP, the overall proportion of heifers with P4 ≥ 1 ng/mL was 84% and differed ($P < 0.01$) among treatments because fewer heifers in the PGF+AIE treatment had P4 ≥ 1 ng/mL than in the PGF+TAI and ALL-TAI treatments (Table 1). We found no difference ($P = 0.20$) in the proportion of cyclic heifers (i.e., at least 1 of the samples with P4 ≥ 1 ng/mL) by the beginning of the AIP (Table 1).

For first service, the total proportion of heifers that received AIE was greater ($P < 0.01$) for the PGF+AIE (98.7%) than for the PGF+TAI (78.5%) treatment. For the PGF+AIE treatment (arithmetic mean reported), 78.5% (248/317) of the heifers received AIE after the first PGF_{2α}, 17.0% (54/317) after the second, and 3.2% (10/317) after the third PGF_{2α} treatment; thus, 1.3% of the heifers in the PGF+AIE treatment received TAI. The proportion of heifers in the PGF+AIE treatment that were AIE after a single PGF_{2α} treatment was similar ($P = 0.49$) to that of heifers AIE after 2 PGF_{2α} treatments in the PGF+TAI treatment (PGF+AIE = 76.1%; PGF+TAI = 78.5%).

The pattern of insemination and interval from the beginning of the AIP to first service was affected ($P <$

Figure 2. Kaplan-Meier survival curves for time to insemination during the artificial insemination period (AIP). The hazard of AI was greater ($P < 0.01$) for the ALL-TAI than the PGF+AIE [hazard ratio (HR) = 1.72; 95% CI = 1.45 to 2.03] and the PGF+TAI (HR = 1.51; 95% CI = 1.28 to 1.77) treatments, but similar for the PGF+AIE and PGF+TAI treatments (HR = 1.14; 95% CI = 0.97 to 1.33). Mean days to first AI were 6.9 ± 0.46 , 5.7 ± 0.31 , and 2.7 ± 0.03 for the PGF+AIE, PGF+TAI, and ALL-TAI treatments, respectively. Treatment at 368 ± 10 d of age, heifers received first AI after (1) PGF+AIE: insemination at detected estrus (AIE) after PGF_{2α} treatments 14 d apart (up to 3) starting at the beginning of the AIP. Heifers not AIE within 9 d of the third PGF_{2α} treatment were enrolled in a 5-d Cosynch protocol [5-d Cosynch = controlled internal drug release insert (CIDR) + GnRH-5 d-CIDR-out + PGF_{2α}-3 d-GnRH + timed artificial insemination (TAI)], (2) PGF+TAI: AIE after the second of 2 PGF_{2α} treatments 14 d apart. Heifers not AIE within 9 d after the second PGF_{2α} were enrolled in 5-d Cosynch, and (3) ALL-TAI: TAI after a 5-d Cosynch protocol. In a subgroup of heifers from all treatment groups ($n = 162$), 2 blood samples were collected 14 d apart to determine circulating concentrations of progesterone.

0.01; Figure 2) by treatment. The hazard of first AI up to 45 d of the AIP was greater for the ALL-TAI than for the PGF+AIE [hazard ratio (HR) = 1.72; 95% CI = 1.45 to 2.03] and PGF+TAI (HR = 1.51; 95% CI = 1.28 to 1.77) treatments, but similar for the PGF+AIE and PGF+TAI treatments (HR = 1.14; 95% CI = 0.97 to 1.33). Mean days to first AI were, 6.9 ± 0.46 , 5.7 ± 0.31 , and 2.7 ± 0.03 for the PGF+AIE, PGF+TAI, and ALL-TAI treatments, respectively.

For all first AI services combined, P/AI 31 ± 3 d after AI did not differ between treatments ($P = 0.38$; Table 2) and was similar ($P = 0.21$) for heifers that received AIE after PGF-synchronized estrus (46.1%; $n = 582$), AIE during 5-d Cosynch (45.7%; $n = 127$), or a TAI (40.1%; $n = 257$) service. In contrast, P/AI for all AIE services combined (46.0%; $n = 709$) tended ($P = 0.10$) to be greater than TAI services (40.1%; $n = 257$). Overall, P/AI was not affected ($P = 0.22$) by season of AI (cold = 43.1%; warm = 47.2%). For AIE services synchronized with PGF_{2α}, P/AI for the

PGF+TAI treatment was greater ($P = 0.03$; Table 2) than for PGF+AIE (52.9% and 44.0%, respectively). In addition, P/AI for heifers AIE in the PGF+TAI treatment (i.e., after 2 PGF_{2α}) tended ($P = 0.06$) to be greater than for heifers AIE after the first PGF_{2α} treatment in the PGF+AIE treatment (PGF+TAI = 53.6% vs. PGF+AIE = 45.2%). In contrast, P/AI for heifers in the PGF+AIE treatment receiving 1 PGF_{2α} treatment were similar ($P = 0.97$) to that of heifers receiving 2 or 3 PGF_{2α} treatments (1 PGF_{2α} = 42.4%; 2 or 3 PGF_{2α} = 42.2%). No difference in P/AI was detected for AIE services during the 5-d Cosynch protocol ($P = 0.86$; Table 2) or for TAI services ($P = 0.13$; Table 2) between the ALL-TAI and PGF+TAI treatments. Out of all first AI services in the ALL-TAI treatment, 33% (104/315) occurred during the protocol (majority between the time of CIDR removal and the scheduled TAI) and P/AI were similar ($P = 0.43$) for AIE (46.5%) and TAI services (41.8%).

Reproductive Outcomes for Second and Greater AI Services

The proportion of heifers AIE before resynchronization of ovulation with the 5-d Cosynch protocol for second and greater services tended ($P = 0.06$) to differ among treatments [PGF+AIE (59.7%), PGF+TAI (66.6%), and ALL-TAI (69.7%)]. Similarly, the proportion of heifers AIE during the 5-d Cosynch protocol differed ($P = 0.02$) among treatments [PGF+AIE (11.2%), PGF+TAI (7.8%), and ALL-TAI (5.7%)]. Overall, P/AI for all second and greater AI services combined was not different among treatments ($P = 0.72$; Table 2) and seasons ($P = 0.60$). Further, treatment did not affect P/AI for heifers that received AIE before ($P = 0.94$) and during resynchronization with 5-d Cosynch ($P = 0.83$) or TAI ($P = 0.58$; Table 2).

Type of insemination affected overall P/AI ($P = 0.02$) because P/AI for AIE services before (59.3%; $n = 531$) and during resynchronization with 5-d Cosynch (65.6%; $n = 96$) were greater than after TAI services (51.4%; $n = 313$). Moreover, P/AI for all AIE combined (60.3%; $n = 627$) was greater than for TAI services (51.4%; $n = 313$). Heifers (all treatment groups) that received AIE between CIDR removal and the expected TAI day (P/AI = 62.3%; $n = 96$) had greater ($P = 0.01$) P/AI than heifers inseminated on the scheduled day of TAI (P/AI = 46.9%; $n = 313$).

Time to Pregnancy and Nonpregnant Heifers at the End of the AIP

The hazard of pregnancy for up to 100 d of AIP was greater for the ALL-TAI than the PGF+AIE treatment

Table 2. Pregnancy per AI (P/AI) 31 ± 3 d after first AI service and after second or greater AI services combined

Item	Treatment, % (n) ¹			P-value
	PGF+AIE	PGF+TAI	ALL-TAI	
Overall P/AI for first AI service ²	42.0 (317)	47.3 (334)	43.8 (315)	0.38
P/AI for AIE after PGF _{2α} ³	44.0 (313)	52.9 (269)	—	0.03
P/AI for AIE during 5-d Cosynch ⁴	—	45.0 (20)	47.1 (104)	0.86
P/AI for TAI ⁵	—	29.0 (45)	41.4 (211)	0.13
Overall P/AI for second and greater AI services ⁶	58.8 (311)	55.7 (318)	57.6 (311)	0.72
P/AI for AIE after AI ⁷	59.1 (154)	58.5 (188)	60.3 (189)	0.94
P/AI for AIE during 5-d Cosynch	62.0 (45)	60.0 (30)	66.9 (21)	0.83
P/AI for TAI	53.6 (112)	47.1 (100)	47.7 (101)	0.58

¹Treatment: at 368 ± 10 d of age, heifers received first AI after (1) PGF+AIE: insemination at detected estrus (AIE) after PGF_{2α} treatments 14 d apart (up to 3) starting at the beginning of the AIP. Heifers not AIE within 9 d of the third PGF_{2α} treatment were enrolled in a 5-d Cosynch protocol [5-d Cosynch = controlled internal drug release insert (CIDR) + GnRH-5 d-CIDR-out + PGF_{2α}-3 d-GnRH + TAI] followed by (2) PGF+TAI: AIE after the second of 2 PGF_{2α} treatments 14 d apart. Heifers not AIE within 9 d after the second PGF_{2α} were enrolled in 5-d Cosynch and (3) ALL-TAI: timed AI after a 5-d Cosynch protocol. In a subgroup of heifers from all treatment groups (n = 162), 2 blood samples were collected 14 d apart to determine circulating concentrations of progesterone.

²Sex-selected semen was used for all first AI services.

³Heifers inseminated at detected estrus after PGF_{2α}-induced estrus.

⁴Heifers inseminated anytime during the 5-d Cosynch protocol. Heifers did not receive GnRH at insemination.

⁵TAI = timed AI. P/AI not included for the PGF+AIE group because n = 4.

⁶Conventional semen was used for all second and greater AI services.

⁷Heifers inseminated at detected estrus after a previous AI service and before initiation of resynchronization with 5-d Cosynch.

(HR = 1.20, 95% CI = 1.02 to 1.42; Figure 3), but was similar for the PGF+AIE and PGF+TAI (HR = 1.07, 95% CI = 0.91 to 1.25) treatments, and for the ALL-TAI and PGF+TAI treatments (HR = 1.13, 95% CI = 0.96 to 1.33). Median days from the beginning of the AIP to pregnancy were 27 (95% CI = 23 to 36), 23 (95% CI = 17 to 26), and 21 (95% CI = 10 to 23) for heifers in the PGF+AIE, PGF+TAI, and ALL-TAI treatment, respectively. Furthermore, mean (±SE) days from the beginning of the AIP to pregnancy were 36 ± 1.8, 32 ± 1.8, and 28 ± 1.7 for heifers in the PGF+AIE, PGF+TAI, and ALL-TAI treatment, respectively. Overall, the proportion of heifers not pregnant the end of the AIP (includes heifers sold and dead during the 100 d) did not differ ($P = 0.97$) for the PGF+AIE (7.0%), PGF+TAI (6.5%), and ALL-TAI (6.8%) treatments.

DISCUSSION

In the present experiment, we evaluated the reproductive performance of dairy heifers managed with

reproductive management strategies that differed on the method of submission to first service. We compared a program that relied primarily on AI after detection of estrus versus a program that relied primarily on TAI. An intermediate program designed to reduce days to first service through a combination of AIE and TAI was also included. In support of our hypothesis, the program that relied primarily on TAI reduced time to pregnancy when compared with the program using predominant AIE after induction of estrus with PGF_{2α} treatments. The reduction of time to pregnancy, which was of a small magnitude, was primarily due to earlier insemination, because P/AI was similar for AIE and TAI services in the PGF+AIE and ALL-TAI groups. Conversely, the ALL-TAI program did not reduce time to pregnancy when compared with the program that combined synchronization of estrus and TAI. A high proportion of heifers AIE within a short time after the beginning of the AIP and high P/AI for these AIE services for the combined approach reduced differences in time to pregnancy between groups. For the PGF+TAI treatment, immediate submission to a TAI protocol

(i.e., within 9 d) rather than extending the period of estrus detection was also important to reduce time to pregnancy. Collectively, our data suggests that ALL-TAI programs for first AI service may be more effective to increase the pregnancy rate of heifers than programs that rely primarily on AIE services after systematic synchronization of estrus with $\text{PGF}_{2\alpha}$. Conversely, ALL-TAI programs may not be more effective than a combined approach (i.e., AIE plus TAI) when estrus detection efficiency immediately after the beginning of the AIP is high, as in our experiment (e.g., ~80% of heifers detected in estrus). Thus, programs that combine AIE and TAI may be an effective strategy to

attain similar reproductive performance than ALL-TAI programs for farms that prefer to maximize inseminations at detected estrus rather than TAI. These combined programs, however, may not be more effective for reducing time to pregnancy than programs that result in high estrus detection efficiency after $\text{PGF}_{2\alpha}$ -induced estrus every 14 d.

Few previous experiments compared insemination and pregnancy dynamics of dairy heifers managed with strategies that rely primarily on TAI versus less-intensive AIE-based reproductive programs. In agreement with our findings, Silva et al. (2015) reported that heifers submitted to first service with a TAI-based program (5-d Cosynch with 2 $\text{PGF}_{2\alpha}$ treatments) were inseminated earlier and had greater pregnancy rate when compared with heifers managed with a program that relied primarily on AIE after spontaneous and $\text{PGF}_{2\alpha}$ -induced estrus. In contrast, Lopes et al. (2013) reported that heifers receiving TAI-based programs (5- or 7-d Cosynch with 1 $\text{PGF}_{2\alpha}$ treatment) had similar (5-d Cosynch) or more (7-d Cosynch) mean days to first AI but delayed time to pregnancy when compared with all AIE after induction of estrus with $\text{PGF}_{2\alpha}$ every 11 d. A key aspect of the experiment reported in Lopes et al. (2013) was that heifers in the AIE treatment became eligible for insemination 7.5 or 9.5 d earlier than heifers in the 5- and 7-d Cosynch programs, respectively. This delay on time to first service imposed by the experimental design was substantial and explained part of the delay on time to pregnancy for the TAI programs. In contrast, in the current experiment and that of Silva et al. (2015), the TAI programs resulted in earlier pregnancy because all heifers were inseminated within either 3 or 2 d of the beginning of the AIP, respectively. Unlike the current experiment and that of Silva et al. (2015), Lopes et al. (2013) also reported a significant reduction in P/AI (16.3 and 25.4 percentage points for 5- and 7-d Cosynch with a single $\text{PGF}_{2\alpha}$ treatment and no initial GnRH) for heifers that received TAI compared with AIE. Thus, matching the beginning of the AIP with the day of TAI or the day of induction of luteolysis and P4 device removal and obtaining similar P/AI for TAI than AIE services seems critical to benefit from TAI programs for dairy heifers.

Implementation of TAI at or near the beginning of the AIP also contributes to reduce time to pregnancy because return to estrus is tightly synchronized, reducing overall timing of reinsemination at detected estrus. Indeed, the greater proportion (statistical tendency) of heifers that received AIE for second and greater AI services for the ALL-TAI group in our experiment may have been, at least in part, the result of tighter synchrony of estrus after previous inseminations.

Figure 3. Kaplan-Meier survival curves for time to pregnancy up to 100 d after the beginning of the artificial insemination period (AIP). The hazard of pregnancy was greater for the ALL-TAI than the PGF+AIE group [hazard ratio (HR) = 1.20; 95% CI = 1.02 to 1.42] but was similar for the ALL-TAI and PGF+TAI (HR = 1.13; 95% CI = 0.96 to 1.32) and the PGF+AIE and PGF+TAI groups (HR = 1.07; 95% CI = 0.91 to 1.25). Median days to pregnancy were 27 (95% CI = 23 to 36) for the PGF+AIE group, 23 for the PGF+TAI group (95% CI = 17 to 26), and 21 for the ALL-TAI group (95% CI = 10 to 23). Further, mean ($\pm$ SE) days from the beginning of the AIP to pregnancy were 36 ± 1.8 , 32 ± 1.8 , and 28 ± 1.7 for heifers in the PGF+AIE, PGF+TAI, and ALL-TAI treatment, respectively. Downward vertical lines on survival curves depict right censoring of heifers due to herd exit (i.e., sale or death). Treatment at 368 ± 10 d of age, heifers received first AI after (1) PGF+AIE: insemination at detected estrus (AIE) after $\text{PGF}_{2\alpha}$ treatments 14 d apart (up to 3) starting at the beginning of the AIP. Heifers not AIE within 9 d of the third $\text{PGF}_{2\alpha}$ treatment were enrolled in a 5-d Cosynch protocol [5-d Cosynch = controlled internal drug release insert (CIDR) + GnRH-5 d-CIDR-out + $\text{PGF}_{2\alpha}$ -3 d-GnRH + timed artificial insemination (TAI)], (2) PGF+TAI: AIE after the second of 2 $\text{PGF}_{2\alpha}$ treatments 14 d apart. Heifers not AIE within 9 d after the second $\text{PGF}_{2\alpha}$ were enrolled in 5-d Cosynch, and (3) ALL-TAI: timed AI after a 5-d Cosynch protocol. In a subgroup of heifers from all treatment groups ($n = 162$), 2 blood samples were collected 14 d apart to determine circulating concentrations of progesterone.

We included 2 PGF_{2α} treatments to synchronize estrus in the PGF+TAI treatment group because synchronization of estrus programs with 2 PGF_{2α} treatments 11 to 14 d apart (i.e., Presynch) ensure that most cyclic heifers or cows have a responsive corpus luteum to PGF_{2α} at the time of the second treatment (King and Robertson, 1974; Stevenson et al., 2000). Ultimately, we expected to hasten overall timing of insemination by inducing a greater proportion of heifers displaying estrus immediately after the beginning of the AIP. The similar proportion of heifers AIE after the first PGF_{2α} of the PGF+AIE treatment group and the second PGF_{2α} of the PGF+TAI treatment, however, did not support our reasoning. It is unlikely that the lack of difference was due to poor estrus detection, because it was conducted equally for both groups; PGF_{2α} treatments were given on the same days of the week and time by the same personnel and heifers were managed and housed in the same pens. In addition, the proportion of heifers AIE in the PGF+TAI group (78.5%) was similar to that reported in a previous experiment for dairy heifers detected in estrus after 2 PGF_{2α} treatments 14 d apart (Colazo and Mapletoft, 2017). Most likely, having a vast majority of heifers cyclic when they became eligible for insemination in the PGF+AIE group resulted in a good response to PGF_{2α} and spontaneous estrus, which led to the lack of difference in proportion of heifers that received AIE.

The greater P/AI for AIE services in the PGF+TAI than the PGF+AIE treatment was unexpected but may be explained, at least in part, by the occurrence of more estrus events before first service (i.e., due to first PGF_{2α} treatment), improved synchrony of ovulation, or both. In lactating dairy cows, it has been shown that more estrus events before first service increase first service P/AI (Thatcher and Wilcox, 1973; Butler and Smith, 1989; Darwash et al., 1997). In addition, Stevenson and Phatak (2005) reported greater P/AI in lactating dairy cows inseminated at detected estrus after 2 rather than after a single PGF_{2α} treatment before the end of the voluntary waiting period. Unlike our experiment, in which heifers were approximately the same age when eligible for first service, DIM at first service may have affected results for P/AI because cows that received 2 PGF_{2α} treatments were inseminated at later DIM, which has been shown to increase P/AI in lactating dairy cows (Stangaferro et al., 2018). Additional research with dairy heifers is needed to confirm if induction of estrus with 2 PGF_{2α} treatments leads to greater P/AI than insemination at detected estrus after a single PGF_{2α} treatment. This is relevant because any potential increase in P/AI must offset the burden (i.e., additional handling) and costs (i.e., labor and PGF_{2α}) of the additional PGF_{2α} treatment.

In the current study, first service inseminations with sex-selected semen at detected estrus during the 5-d Cosynch protocol led to similar P/AI than inseminations after PGF_{2α}-induced estrus or TAI. On the other hand, for second and subsequent AI services with conventional semen, inseminations during the protocol resulted in similar P/AI than after spontaneous estrus, but greater P/AI than after TAI. Thus, immediate insemination without GnRH treatment of heifers that display estrus before the scheduled TAI seems a reasonable strategy to maximize overall P/AI with synchronization of ovulation programs. Obtaining similar or greater P/AI with TAI programs than other methods of submission to insemination is critical to benefit from predominant TAI programs that hasten insemination immediately after the beginning of the AIP. Otherwise, delayed pregnancy may be observed (Lopes et al., 2013).

The choice of a synchronization of ovulation protocol for TAI in commercial dairy operations depends primarily on the balance between optimizing P/AI and the constraints of protocol implementation. In our experiment, including GnRH at CIDR insertion and a single PGF_{2α} treatment in the 5-d Cosynch protocol was primarily due to the simplicity of giving GnRH at CIDR insertion and the logistical difficulty of adding a second PGF_{2α} treatment. Although the protocol used may not have optimized the physiological response of heifers to the protocol due to a greater risk for incomplete luteolysis in heifers that ovulated in response to GnRH (Lima et al., 2013), the effect on P/AI is difficult to estimate because results from previous experiments have been inconclusive. Some experiments reported that 2 PGF_{2α} treatments were necessary to maximize P/AI when the initial GnRH treatment was given to dairy heifers (Kasimanickam et al., 2009; Lima et al., 2013), whereas others reported no differences in P/AI for both dairy and beef heifers that received GnRH and a single PGF_{2α} treatment (Rabaglino et al., 2010; Kasimanickam et al., 2014).

Semen type (i.e., sex-selected vs. conventional) plays a major role on defining P/AI (Chebel et al., 2010), possibly contributing to differences observed in P/AI and pregnancy dynamics among different studies. The ratio of P/AI for sex-selected to conventional semen that we observed (78 and 76% of that obtained with conventional semen for AIE and TAI services, respectively) is similar to that previously reported for commercial dairy farms in the United States (Norman et al., 2010) and for heifers randomized to insemination for first service with sex-selected or conventional semen (Chebel et al., 2010). Therefore, results of the current experiment for P/AI and pregnancy dynamics should be interpreted within the context of type of semen used. Our results may better reflect the conditions of

commercial farms that use sex-selected semen for first service. Conversely, the expected performance of the treatments evaluated on farms that use conventional semen only may differ substantially, as greater P/AI for first service may dramatically change the pregnancy dynamics.

Finally, an important consideration for the use of the intensive programs for synchronization of estrus and TAI (e.g., ALL-TAI and PGF+TAI in our experiment) for dairy heifers is their effect on the profitability of the heifer enterprise. Reducing time to pregnancy shortens the nonlactating period of a dairy animal, which, in turn, results in fewer days on feed during the rearing period and favors an earlier positive cash flow via milk production. Therefore, for intensive programs including TAI to be more profitable than primarily AIE programs, the expected reduction in time to pregnancy must offset the associated additional labor and treatment costs. In this regard, previous economic comparisons of more intensive TAI and AIE programs suggested that differences in costs and revenues were strongly associated with the observed reproductive performance, but also depended on the type of inputs and the methods used to quantify the benefit of earlier pregnancy (Lopes et al., 2013; Silva et al., 2015). The economic effect of managing heifers with the reproductive management strategies compared in our experiment warrants further investigation and will be reported in a separate manuscript.

CONCLUSIONS

Data from the present experiment demonstrated that a reproductive management strategy for dairy heifers designed to submit heifers to TAI at or near the beginning of the AIP resulted in reduced time to first AI and reduced time to pregnancy when compared with a strategy that resulted in almost all inseminations after induction of estrus with PGF_{2α} treatments every 14 d. Nonetheless, results of our study support the notion that, in herds with effective estrus detection programs, the combination of AIE and TAI may result in similar reproductive performance than with programs that rely mostly on TAI. Thus, dairy farms have the option to either select a strategy that reduces days to first service through TAI or a combined strategy that attempts to maximize AIE but includes a TAI protocol immediately after completion of a short period of estrus detection. Additional research is needed to determine the effect of the different reproductive management strategies evaluated on the economic performance of the dairy heifer enterprise.

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REFERENCES

- Abramson, J. H. 2011. WINPEPI updated: Computer programs for epidemiologists, and their teaching potential. *Epidemiol. Perspect. Innov.* 8:1.
- Butler, W. R., and R. Smith. 1989. Interrelationships between energy balance and postpartum reproductive function in dairy cattle. *J. Dairy Sci.* 72:767–783.
- Caraviello, D. Z., K. Weigel, P. Fricke, M. Wiltbank, M. Florent, N. Cook, K. Nordlund, N. Zwald, and C. Rawson. 2006. Survey of management practices on reproductive performance of dairy cattle on large US commercial farms. *J. Dairy Sci.* 89:4723–4735.
- Chebel, R. C., F. Guagnini, J. Santos, J. Fetrow, and J. Lima. 2010. Sex-sorted semen for dairy heifers: Effects on reproductive and lactational performances. *J. Dairy Sci.* 93:2496–2507.
- Chebel, R. C., J. E. Santos, R. L. Cerri, H. M. Rutigliano, and R. Bruno. 2006. Reproduction in dairy cows following progesterone insert presynchronization and resynchronization protocols. *J. Dairy Sci.* 89:4205–4219.
- Colazo, M. G., and R. J. Mapletoft. 2017. Pregnancy per AI in Holstein heifers inseminated with sex-selected or conventional semen after estrus detection or timed-AI. *Can. Vet. J.* 58:365.
- Darwash, A., G. Lamming, and J. Wooliams. 1997. The phenotypic association between the interval to post-partum ovulation and traditional measures of fertility in dairy cattle. *Anim. Sci.* 65:9–16.
- Ettema, J. F., and J. Santos. 2004. Impact of age at calving on lactation, reproduction, health, and income in first-parity Holsteins on commercial farms. *J. Dairy Sci.* 87:2730–2742.
- Ferguson, J. D., and A. Skidmore. 2013. Reproductive performance in a select sample of dairy herds. *J. Dairy Sci.* 96:1269–1289.
- Fricke, P. M., J. Giordano, A. Valenza, G. Lopes, M. Amundson, and P. Carvalho. 2014. Reproductive performance of lactating dairy cows managed for first service using timed artificial insemination with or without detection of estrus using an activity-monitoring system. *J. Dairy Sci.* 97:2771–2781.
- Garbarino, E. J., J. Hernandez, J. Shearer, C. Risco, and W. Thatcher. 2004. Effect of lameness on ovarian activity in postpartum Holstein cows. *J. Dairy Sci.* 87:4123–4131.
- Giordano, J. O., M. Thomas, G. Catucuaamba, M. Curler, R. Wijma, M. Stangaferro, and M. Masello. 2016. Effect of extending the interval from Presynch to initiation of Ovsynch in a Presynch-Ovsynch protocol on fertility of timed artificial insemination services in lactating dairy cows. *J. Dairy Sci.* 99:746–757.
- Higgs, R. J., L. E. Chase, D. A. Ross, and M. E. Van Amburgh. 2015. Updating the Cornell Net Carbohydrate and Protein System feed library and analyzing model sensitivity to feed inputs. *J. Dairy Sci.* 98:6340–6360.
- Kasimanickam, R., M. Day, J. Rudolph, J. Hall, and W. Whittier. 2009. Two doses of prostaglandin improve pregnancy rates to timed-AI in a 5-day progesterone-based synchronization protocol in beef cows. *Theriogenology* 71:762–767.
- Kasimanickam, R. K., P. Firth, G. M. Schuenemann, B. Whitlock, J. Gay, D. Moore, J. Hall, and W. Whittier. 2014. Effect of the first GnRH and two doses of PGF_{2α} in a 5-day progesterone-based CO-Synch protocol on heifer pregnancy. *Theriogenology* 81:797–804.

- King, G. J., and H. A. Robertson. 1974. A two injection schedule with prostaglandin $F_{2\alpha}$ for the regulation of the ovulatory cycle of cattle. *Theriogenology* 1:123–128.
- Lima, F. S., E. Ribeiro, R. Bisinotto, L. Greco, N. Martinez, M. Amstalden, W. Thatcher, and J. Santos. 2013. Hormonal manipulations in the 5-day timed artificial insemination protocol to optimize estrous cycle synchrony and fertility in dairy heifers. *J. Dairy Sci.* 96:7054–7065.
- Lopes, G., Jr., C. Johnson, L. Mendonça, P. Silva, J. Moraes, A. Ahmadzadeh, J. Dalton, and R. Chebel. 2013. Evaluation of reproductive and economic outcomes of dairy heifers inseminated at induced estrus or at fixed time after a 5-day or 7-day progesterone insert-based ovulation synchronization protocol. *J. Dairy Sci.* 96:1612–1622.
- NAHMS. 2018. Dairy 2014, Part III: Reference of Dairy Cattle Health and Management Practices in the United States, 2014. #696.0218. Centers for Epidemiology and Animal Health, USDA, Animal and Plant Health Inspection Service, Veterinary Services, Fort Collins, CO.
- Norman, H. D., J. Hutchison, and R. Miller. 2010. Use of sexed semen and its effect on conception rate, calf sex, dystocia, and stillbirth of Holsteins in the United States. *J. Dairy Sci.* 93:3880–3890.
- Pursley, J. R., M. Wiltbank, J. Stevenson, J. Ottobre, H. Garverick, and L. Anderson. 1997. Pregnancy rates per artificial insemination for cows and heifers inseminated at a synchronized ovulation or synchronized estrus. *J. Dairy Sci.* 80:295–300.
- Rabaglino, M. B., C. Risco, M.-J. Thatcher, I. Kim, J. Santos, and W. Thatcher. 2010. Application of one injection of prostaglandin $F_{2\alpha}$ in the five-day Co-Synch+ CIDR protocol for estrous synchronization and resynchronization of dairy heifers. *J. Dairy Sci.* 93:1050–1058.
- Ribeiro, E. S., A. Monteiro, F. Lima, H. Ayres, R. Bisinotto, M. Favoreto, L. Greco, R. Marsola, W. Thatcher, and J. Santos. 2012. Effects of presynchronization and length of proestrus on fertility of grazing dairy cows subjected to a 5-day timed artificial insemination protocol. *J. Dairy Sci.* 95:2513–2522.
- Schmitt, E. J., T. Diaz, M. Drost, and W. Thatcher. 1996. Use of a gonadotropin-releasing hormone agonist or human chorionic gonadotropin for timed insemination in cattle. *J. Anim. Sci.* 74:1084–1091.
- Silva, T. V., F. Lima, W. Thatcher, and J. Santos. 2015. Synchronized ovulation for first insemination improves reproductive performance and reduces cost per pregnancy in dairy heifers. *J. Dairy Sci.* 98:7810–7822.
- Skenandore, C. S., A. Pineda, J. Bahr, A. Newell-Fugate, and F. Cardoso. 2017. Evaluation of a commercially available radioimmunoassay and enzyme immunoassay for the analysis of progesterone and estradiol and the comparison of two extraction efficiency methods. *Domest. Anim. Endocrinol.* 60:61–66.
- Stangaferro, M. L., R. Wijma, M. Masello, M. J. Thomas, and J. Giordano. 2018. Extending the duration of the voluntary waiting period from 60 to 88 days in cows that received timed artificial insemination after the Double-Ovsynch protocol affected the reproductive performance, herd exit dynamics, and lactation performance of dairy cows. *J. Dairy Sci.* 101:717–735.
- Stevenson, J. S. 2011. Alternative programs to presynchronize estrous cycles in dairy cattle before a timed artificial insemination program1. *J. Dairy Sci.* 94:205–217.
- Stevenson, J. S., and A. Phatak. 2005. Inseminations at estrus induced by presynchronization before application of synchronized estrus and ovulation. *J. Dairy Sci.* 88:399–405.
- Stevenson, J. L., J. Rodrigues, F. Braga, S. Bitente, J. Dalton, J. Santos, and R. Chebel. 2008. Effect of Breeding Protocols and Reproductive Tract Score on Reproductive Performance of Dairy Heifers and Economic Outcome of Breeding Programs1. *J. Dairy Sci.* 91:3424–3438.
- Stevenson, J. S., J. Smith, and D. Hawkins. 2000. Reproductive outcomes for dairy heifers treated with combinations of prostaglandin $F_{2\alpha}$, norgestomet, and gonadotropin-releasing hormone. *J. Dairy Sci.* 83:2008–2015.
- Thatcher, W. W., and C. Wilcox. 1973. Postpartum estrus as an indicator of reproductive status in the dairy cow. *J. Dairy Sci.* 56:608–610.