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Targeted reproductive management for lactating Holstein cows: Reproductive and economic outcomes of Double-Ovsynch compared with a targeted approach based on resumption of estrus

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ABSTRACT

Accessibility to automated monitoring devices (AMD) has led to exploration of alternative reproductive management to ovulation synchronization protocols (OvSP) for first postpartum artificial insemination (AI) according to the cow's early postpartum estrus characteristics (EPEC). We hypothesized that pregnancy and economic outcomes of cows subjected to a targeted reproductive management (TRM) are not inferior to those of cows subjected to an OvSP for the first AI. This was a noninferiority, randomized clinical trial. Cows (n = 2,635) from one dairy were fitted with AMD and classified according to EPEC at 45 ± 3 DIM as estrual (high intensity AMD-detected estrus [primiparous: heat index ≥90, multiparous: heat index ≥70; 0 = minimum, 100 = maximum]) and anestrus (no estrus or low intensity estrus). Cows in the control treatment were enrolled in the Double-Ovsynch (GnRH on d −27, $PGF_{2\alpha}$ on d −20, GnRH on d −17 and −10, $PGF_{2\alpha}$ on d −3 and −2, GnRH on d −1, and timed AI [TAI] on d 0 at 73 ± 3 DIM). Anestrus cows enrolled in the TRM treatment were assigned to the hCG-Ovsynch (TRM1; hCG on d −17, GnRH on d −10, $PGF_{2\alpha}$ on d −3 and −2, GnRH on d −1, and TAI on d 0 at 73 ± 3 DIM). Estrual cows received $PGF_{2\alpha}$ at 60 to 73 DIM, when they were 6 to 22 d after a previous estrus, and if not AI in estrus within 7 d, were enrolled in the hCG-Ovsynch at 70 to 77 DIM (TRM2). Estrual cows in the TRM treatment that were ≥23 d from a previous estrus at 63 ± 3 DIM were enrolled in the hCG-Ovsynch at 63 ± 3 DIM and received TAI at 80 ± 3 DIM (TRM3). Pregnancy was diagnosed 32 ± 3 and 67 ± 3 d after AI. Cows were re-inseminated at AMD-detected estrus or at fixed time within 10 d after nonpregnancy diagnosis. The lactation gross profit was calculated as follows: (milk income + sale value + subsequent lactation calf value) − (feed cost + replacement cost + fixed cost + depreciation + reproductive management cost). Cows in the control treatment were more likely to be diagnosed pregnant 67 d after AI (control = 53.9% [95% CI = 51.1%, 56.6%]; TRM = 50.1% [95% CI = 47.2%, 53.0%]), independent of EPEC. The interaction between treatment and EPEC tended to affect the hazard of pregnancy throughout the lactation (control = referent; anestrus-TRM: adjusted hazard ratio = 1.01, 95% CI = 0.91, 1.13; estrual-TRM: adjusted hazard ratio = 0.83, 95% CI = 0.74, 0.94). Treatment did not affect gross profit, independent of EPEC (control = US$2,196.9 ± 25.6; TRM = US$2,221.9 ± 26.5). Alternative strategies for first postpartum AI according to a cow's EPEC may be possible with AMD, without affecting gross profit. The use of a single hCG treatment to presynchronize the estrous cycle of anestrus cows may be an alternative to the presynchronization with the Ovsynch protocol because despite slightly decreasing P/AI, it did not affect gross profit.
Key words: targeted reproductive management, human chorionic gonadotropin, automated monitoring device, stochastic model

INTRODUCTION

Effective reproductive management is crucial for dairy farming, as it affects both productivity and profitability. Its primary objective is ensuring cows become pregnant at an interval after calving that helps maintain efficiency in milk production, minimizing lesser productive periods. Effective reproductive management hinges on 4 key factors: (1) timely artificial insemination (**AI**) after the voluntary waiting period, (2) increasing pregnancy per AI (**P/AI**), (3) timely re-AI of nonpregnant cows, and (4) reducing pregnancy losses. These metrics determine

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the 21-d pregnancy rate, which has been tightly linked to economic performance of dairy herds (Cabrera, 2012; Giordano et al., 2012a; Galvão et al., 2013).

Considering the economic importance of reproductive performance for dairy herds, much effort has been made in the past 30 years to improve reproductive performance, from adequation of environment and improvement of nutritional strategies to the development of ovulation synchronization protocols (**OvSP**). These changes have improved peripartum health and led to a shift in reproductive management that all but eliminated the need for estrus detection, particularly for first postpartum AI. Although developed in the 1970s, automated monitoring devices (**AMD**) that detect cows in estrus by identifying behavior pattern changes have had greater penetration in the United States in the past 15 years. Researchers have explored the use of such AMD to understand the importance of early postpartum estrus characteristics (**EPCP**) on future reproductive performance and the development of targeted reproductive management (**TRM**) to tailor hormonal treatment according to the cow's needs. Borchardt et al. (2021) reported that cows with 2 AMD-detected estrus events by 40 DIM had ~25% higher P/AI to first service and ~14 fewer days open than cows with fewer estrus events. Among cows receiving the first service at AMD-detected estrus, those that exhibited estrus before the end of the voluntary waiting period had P/AI 15% higher than those that did not and the former had 15 fewer days open (Rial et al., 2022). This led to the development of TRM, in which the adoption of exogenous reproductive hormones is selective and dependent on the cow's early postpartum estrus characteristics (**EPEC**). Fricke et al. (2014) and Rial et al. (2022) compared the P/AI of cows subjected to the Presynch-Ovsynch or Double-Ovsynch, regardless of EPEC, to that of cows that were allowed varying intervals after the end of the voluntary waiting period to be inseminated in estrus according to their EPEC and enrolled in the OvSP if not AI in estrus. Although in both instances the P/AI of cows receiving timed AI (**TAI**) irrespective of EPEC was greater than those AI at AMD-detected estrus and TAI, the interval from calving to pregnancy was not different between treatments (Fricke et al., 2014) or greater for cows AI at AMD-detected estrus and TAI (Rial et al., 2022). Gonzalez et al. (2023) demonstrated that a TRM that favored AI of cows in estrus reduced the use of hormones over the lactation by nearly 60% and, despite decreasing P/AI to first service among primiparous cows, resulted in calving to pregnancy interval slightly shorter for the TRM. Recent experiments have demonstrated that the adoption of TRM through which the reproductive management of cows for first AI is based on EPEC is a possibility and could reduce the use of reproductive hormones. Thus, we hypothesized that a TRM based on EPEC reduces the use of exogenous reproductive hormones for first postpartum AI while resulting in P/AI that is not inferior to that of cows metaphylactically treated with the Double-Ovsynch protocol. Furthermore, we hypothesized that the economic return of cows in a TRM based on EPEC would not differ from the economic return of cows assigned to the Double-Ovsynch.

Since the inception of OvSP with the advent of the Ovsynch (Pursley et al., 1995), investigators have searched for alternatives to presynchronize the estrus cycle. This is a key step to ascertain that the OvSP is initiated between 5 and 9 d of the estrous cycle to synchronize the recruitment of a new follicular wave (Vasconcelos et al., 1999), elicit the growth of the ovulatory follicle under high progesterone (Denicol et al., 2012), and synchronize luteolysis and ovulation (Pursley et al., 1995). Strategies for presynchronization of the estrus cycle have relied on sequential injections of $PGF_{2\alpha}$ (Moreira et al., 2001; Galvão et al., 2007) or sequential injections of GnRH and $PGF_{2\alpha}$ (Bello et al., 2006; Souza et al., 2008) before the start of the Ovsynch protocol. These presynchronization strategies produced greater P/AI than the Ovsynch alone, with varying degrees of success. On farm success may vary further because of differences in environmental conditions, management, health, and compliance. In fact, Galvão et al. (2013) demonstrated through simulations that in herds that adopt the Presynch-Ovsynch (Moreira et al., 2001) and 100% TAI for first service a decrease in compliance of hormonal treatment from 95% to 85% would decrease P/AI to first service by nearly 25%. Therefore, simplifying the presynchronization protocols through the use of one instead of a sequence of injections could reduce compliance issues and losses in P/AI following TAI. The lack of ovulation in response to the GnRH injection at the start of OvSP causes a prolongment of the dominance period and reduced embryo quality (Cerri et al., 2009) leading to reduced P/AI (Chebel et al., 2006). Compared with GnRH, human chorionic gonadotropin (**hCG**) is a more potent ovulation inducer because it binds directly to LH receptors in the follicles (Ireland and Roche, 1982, 1983). Giordano et al. (2012b) demonstrated that presynchronization of the estrus cycle of cows that had been previously AI with one injection of hCG 7 d before the start of the OvSP significantly increase P/AI compared with no presynchronization. In fact, cows that had their estrus cycle presynchronized with hCG and the Ovsynch protocol had similar P/AI (Giordano et al., 2012b). Thus, we hypothesized that among anestrus cows (no AMD-detected estrus by 41 DIM) and cows with low intensity estrus by 41 DIM (heat index: primiparous <90, multiparous <70; minimum = 0, maximum = 100), the presynchronization of the estrus cycle with one injection of hCG would produce P/AI to first service that is not inferior to the P/AI produced by the presynchronization with the Ovsynch protocol. Although hCG is nearly US$4.75/dose more expensive than the Ovsynch protocol, we hypothesized that similar reproductive performance over the lactation and the decrease number of hormonal treatments and labor associated with presynchronization of the estrus cycle with one injection of hCG would result in economic return over the lactation that is not inferior to presynchronization with the Ovsynch protocol.

The objectives of this study were to evaluate whether the P/AI of cows subjected to a TRM for first AI would not be inferior to that of cows subjected to the Double-Ovsynch. In addition, we aimed to compare the effects of a TRM for first postpartum on long-term reproductive and economic performances of Holstein dairy cows. Furthermore, our objective was to characterize differences in progesterone concentration and ovulation during the OvSP of cows presynchronized with hCG and Ovsynch.

MATERIALS AND METHODS

Animals, Housing, and Management

Cows from one commercial dairy farm located in central Georgia comprising 2 sites (herds A and B) located 8.9 km apart and calving from August to October 2022 were enrolled in the experiment. All animals were housed in herd A from prepartum (i.e., pregnant nulliparous heifers and dry parous cows) until 5 to 20 d postpartum. Thereafter, primiparous cows remained in herd A and multiparous cows either remained in herd A or were moved to herd B according to space availability. Cows in both herds were milked thrice daily. All cows were housed in 6-row, naturally ventilated, sand-bedded, freestall barns. Pens were equipped with fans and sprinklers over the feed troughs and fans over the stalls. An average of 6,670 (70% primiparous, 30% multiparous) and 6,055 (100% multiparous) Holstein cows were milked in herds A and B, respectively, during the experiment. The rolling average milk yield of primiparous and multiparous cows were 35.8 and 44.9 kg/d, respectively, during the experiment. Cows were fed a TMR twice daily designed to meet or exceed their nutritional needs based on their milk yield (NRC, 2001). The main components of the diets were corn silage, alfalfa hay, wheat haylage, corn meal, soybean meal, cottonseed, and a mineral blend. The herd's management software used for data collection was Dairy Comp 305 (Valley Ag Software, Tulare, CA).

All cows were weighed (AP800, Tru-Test Group, Mineral Wells, TX) by herd personnel 3 to 12 h after calving and data referent to BW at calving was retrieved from the on-farm software. At calving, all first lactation cows were fitted with AMD (Merck Animal Health, De Soto, KS), which were kept on the cows until they left the herd (death or sale). A total of 3,080 cows that calved during the enrollment period (88.5%) were genotyped using the 50k SNP (STGenetics, Navasota, TX). Data regarding genomic estimated breeding values for net merit, milk, daughter pregnancy rate (**gDPR**), and cow conception rate were retrieved from the cows that calved during the enrollment period.

Daily weather data were collected from the Albany, Georgia (elevation = 61.9 m, 31.58°N, 84.15°W; ~96 km south of herds A and B) Weather Underground (https://www.wunderground.com/) laboratories. Using the daily average temperature and humidity, we calculated daily average temperature-humidity index (**THI**; Mader et al., 2006):

$$\begin{aligned}\text{THI} = \{&(0.8 \times \text{temperature in °C})\\ &+ [\text{relative humidity in \%}\\ &\times (\text{temperature in °C} - 14.4)] + 46.4\}.\end{aligned}$$

To account for the effect of environmental conditions, we calculated the number of days within 56 d of the event of interest (lactation performance: calving date; pregnancy outcomes: date of AI) that cows were exposed to THI ≥68.

All cows were monitored daily for retained fetal membranes, metritis, mastitis, ketosis, displaced abomasum, digestive disorder, and lameness. All primiparous cows were examined by transrectal palpation of the uterus at 5 DIM for diagnosis of metritis by herd personnel trained by the herd veterinarian. Cows with health index <80 based on the AMD (minimum = 0, maximum = 100) were also examined by herd personnel trained by the herd veterinarian. Failure of detachment of the fetal membranes within 24 h postpartum characterized retained fetal membranes. Metritis was characterized by the presence of fetid, brownish, watery discharge within 21 DIM. Mastitis was defined as abnormal milk (i.e., serous milk or presence of clots, blood, or pus). Cows that had reduced rumination time and, based on visual inspection, decreased appetite upon return from the milking parlor had their urine tested for acetoacetate (KetoStix; Bayer Diagnostics, Tarrytown, NY) and those with ≥moderate ketone bodies in urine were classified as ketotic. Cows with a metallic sound upon percussion and auscultation of the left or right side of the abdomen were diagnosed with displaced of abomasum, which was confirmed by laparoscopy. Cows with dry scant manure, lack of appetite, and rumen stasis were classified as having a digestive disorder. Individual daily milk yield was recorded and stored on the on-farm software and the daily average in the first 4 wk postpartum was calculated for each cow. Data regarding calf sex, calving difficulty (1 = unassisted parturition, 2 = minimal assistance, 3 =

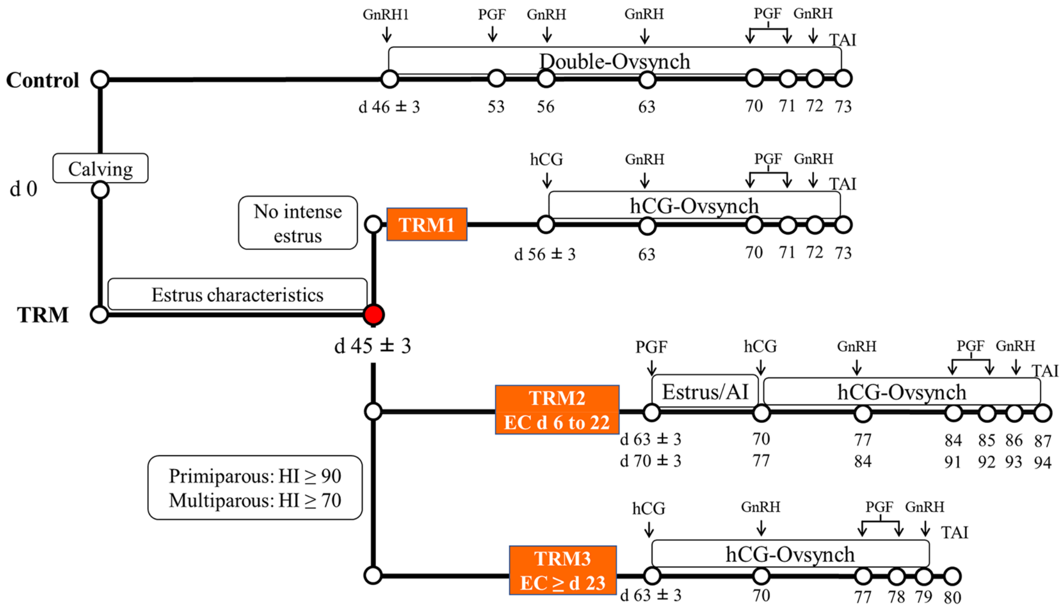

Figure 1. Design of experiment. First postpartum AI. Control: Cows subjected to the Double-Ovsynch protocol (Souza et al., 2008) at 46 ± 3 DIM for first postpartum AI at fixed time (86 µg of gonadorelin at 46 ± 3 DIM, 500 µg of cloprostenol sodium at 53 ± 3, 86 µg of gonadorelin at 56 ± 3 and 63 ± 3 DIM, 500 µg of cloprostenol sodium at 70 ± 3 and 71 ± 3 DIM, 86 µg of gonadorelin at 72 ± 3 DIM, fixed-time AI at 73 ± 3 DIM). TRM: TRM1 = cows without an automated monitoring device (AMD) detected estrus with high heat index (HI; primiparous ≥90, multiparous ≥70; 0 = minimum, 100 = maximum) by 45 ± 3 DIM were enrolled in the hCG-Ovsynch (2,000 IU of human chorionic gonadotropin at 56 ± 3 DIM and the Ovsynch protocol 7 d later [86 µg of gonadorelin at 63 ± 3 DIM, 500 µg of cloprostenol sodium at 70 ± 3 and 71 ± 3 DIM, 86 µg of gonadorelin at 72 ± 3 DIM, fixed-time AI at 73 ± 3 DIM]). TRM2 = Cows with at least one high heat index by 45 ± 3 DIM were monitored and received a $PGF_{2\alpha}$ treatment at 60 to 73 DIM, when they were 6 to 22 d after estrus. Cows were inseminated in estrus for 7 d and those not detected in estrus were enrolled in the hCG-Ovsynch, 67 to 80 DIM. TRM3 = cows that had had a high intensity estrus by 45 ± 3 DIM but where ≥23 d after estrus at 63 ± 3 DIM were enrolled in the hCG-Ovsynch. All cows were re-inseminated upon detection of estrus by an AMD.

assistance; 4 = severe assistance; 5 = cesarean section or fetotomy), calving problems (twins, stillbirth, dystocia), clinical diseases, and milk yield were collected from the on-farm software.

Experimental Design

During the enrollment period (August 19, 2022, to October 27, 2022), 1,167 and 2,315 primiparous and multiparous cows calved in the collaborating herd. At 45 ± 3 DIM, 999 primiparous and 1,890 multiparous cows were enrolled. Cows were blocked by parity (primiparous vs. multiparous) and multiparous cows were ordered by previous lactation 305-d milk yield. Randomization was accomplished by using the "RANDBETWEEN" function of Excel (Microsoft, Redmond, WA).

Experimental design is depicted in Figure 1. Cows assigned to the control treatment were enrolled in the Double-Ovsynch protocol at 46 ± 3 DIM. As such, cows were treated with GnRH at 46 ± 3 DIM (d −27), $PGF_{2\alpha}$ on d −20, GnRH on d −17 (56 ± 3 DIM) and −10, $PGF_{2\alpha}$ on d −3 and −2, GnRH on d 0, and TAI on d 17 (73 ± 3 DIM). Cows enrolled in the TRM treatment were assigned to synchronization protocols according to their EPEC recorded by the AMD up to 45 ± 3 DIM:

1. **TRM1**: cows that did not have an AMD-detected estrus with high heat index (primiparous ≥90, multiparous ≥70; 0 = minimum, 100 = maximum) by 45 ± 3 DIM were enrolled in the hCG-Ovsynch (hCG at 56 ± 3 DIM, GnRH at 63 ± 3 DIM, $PGF_{2\alpha}$ at 70 ± 3 and 71 ± 3 DIM, GnRH at 72 ± 3 DIM, TAI at 73 ± 3 DIM);
2. **TRM2**: cows with at least one high heat index AMD-detected estrus by 45 ± 3 DIM received a $PGF_{2\alpha}$ treatment at 60 to 73 DIM when they were 6 to 22 d after a previously recorded estrus. Cows were monitored for signs of estrus for 7 d after the $PGF_{2\alpha}$ treatment; cows detected in estrus were AI on the same day, whereas those not detected in estrus were enrolled in the hCG-Ovsynch 67 to 80 DIM; and,
3. **TRM3**: cows that had had at least one high heat index AMD-detected estrus by 45 ± 3 DIM but where ≥23 d from a previously recorded estrus at 63 ± 3 DIM were enrolled in the hCG-Ovsynch at 63 ± 3 DIM.

The thresholds used for classification of high heat index (primiparous ≥90, multiparous ≥70) were based on the experiment carried out by Gonzalez et al. (2023),

Table 1. Monetary value of variables and labor allocation recorded in the collaborating herd during the experiment and BW estimated at sale according to lactation group

Output	Value	Input	Value
Milk price, $/kg	0.47	Feed cost, $/kg DM	
Cow sale value, $/kg live BW	2.51	Lactating cows	0.32
BW at calving by lactation group, kg (± SD)		Dry cows	0.27
1	650.9 ± 68.6	Fixed cost, $/day	
2	707.8 ± 64.2	Lactating cows	3.50
3	759.5 ± 69.6	Dry cows	2.00
4	770.3 ± 74.0	Depreciation, $/day	0.65
5	777.5 ± 72.7	Replacement cost, $/cow	2,000
≥6	759.7 ± 64.8	Herdsperson labor, $/h	15
Calf value, $/calf		Semen cost, $/dose	
Female Holstein	100	Sex-sorted Holstein	18
Male Holstein	80	Beef	6
Crossbred	350	Artificial insemination	
Estimated BW at sale by lactation group, kg (± SD)		Supplies, $/AI	0.50
1	498.7 ± 60.6	Time, AI/h	30
2	626.0 ± 70.6	Automated devices	
3	721.5 ± 78.6	Daily fee, $/cow	0.09
4	740.9 ± 77.8	Time to fit AMD,[1] cows/h	30
5	754.0 ± 84.4	Reproductive hormone	
≥6	741.8 ± 66.3	GnRH, $/dose	1.75
		$PGF_{2\alpha}$, $/dose	1.75
		Supplies, $/dose	0.50
		Time, dose/h	60
		Reproductive exams	
		Time, exams/h	30
		Veterinary services, $/h	150

[1]AMD = automated monitoring device.

in which we demonstrated that although the heat index ≥70 was adequate for multiparous cows, it was too low for primiparous cows, resulting in reduced P/AI to the first AI. Hormonal treatments consisted of GnRH (86 μg of gonadorelin; Fertagyl, Merck Animal Health, De Soto, KS), $PGF_{2\alpha}$ (500 μg of cloprostenol sodium; Estrumate, Merck Animal Health, De Soto, KS), and hCG (2,000 IU of human chorionic gonadotropin; Chorulon, Merck Animal Health, De Soto, KS).

All cows were eligible to be re-inseminated at AMD-detected estrus. Cows that were not re-inseminated were treated with GnRH 26 ± 3 d after AI and those diagnosed as nonpregnant by transrectal ultrasonography (Easi-Scan; Technology Ltd., Livingston, UK) 33 ± 3 d after AI were enrolled in the Ovsynch protocol (d 0, GnRH; d 7 and 8, $PGF_{2\alpha}$; d 9, GnRH; d 10, TAI). Pregnant cows were re-examined 66 ± 3 d after AI.

Exclusion criteria were: cows deemed not eligible for AI by the herd manager before the first AI, cows removed from the herd (sold or died) before the first AI, and cows that received incorrect treatment according to the experimental design. Data regarding the sire of AI, AI technician, and reproductive outcomes were collected from the on-farm software. Cows detected in estrus by the AMD were AI according to the recommendations of the manufacturer (Allflex Livestock Intelligence, Madison, WI). Sire selection for AI was based on parity and genomic estimated breeding value for net merit. Among primiparous cows retained in the experiment (n = 939), breed of sires used in 60.0%, 32.7%, and 8.3% of first AI of control cows was Holstein (sex-sorted), Limousin, and Angus, respectively, whereas 55.8%, 32.9%, and 11.3% of first AI of TRM cows were Holstein (sex-sorted), Limousin, and Angus, respectively. The breed of sires used for the re-AI of primiparous cows was 3.4% Holstein (sex-sorted), 72.0% Limousin, and 24.6% Angus among control cows, and 1.9% Holstein (sex-sorted), 71.7% Limousin, and 26.4% Angus among TRM cows. Among multiparous cows retained in the experiment (n = 1,696), breed of sires used in 10.5%, 47.6%, and 41.9% of first AI of control cows was Holstein (sex-sorted), Limousin, and Angus, respectively, whereas 10.9%, 52.3%, and 36.8% of first AI of TRM cows were Holstein (sex-sorted), Limousin, and Angus, respectively. The breed of sires used for the re-AI of multiparous cows was 62.1% Limousin and 37.9% Angus among control cows, and 63.6% Limousin and 36.4% Angus among TRM cows.

Ultrasonography of the Ovaries, Presumptive Ovulation, and Progesterone Concentrations

A subsample of anestrus cows (control = 88, TRM1 = 106) had ovaries examined by ultrasonography (Easi-Scan; Technology Ltd., Livingston, UK) at the time of the first GnRH and $PGF_{2\alpha}$ injections of the Ovsynch protocol. The number of corpora lutea and number of

Table 2. Monetary value of variables used in the Monte Carlo stochastic analyses of economic return

Output	Value ± SD (dist.: min., max.)[1]	Input	Value ± SD (dist.: min., max.)[1]
Milk price,[2] \$/kg	0.50 ± 0.03 (N: 0.37, 0.63)	Feed cost,[4] \$/kg of DM	
Cow sale value[3], \$/kg of live BW	1.65 ± 0.12 (N: 1.15, 2.17)	Lactating	0.30 ± 0.04 (N: 0.14, 0.47)
Calf value,[3] \$/calf		Dry	0.25 ± 0.04 (N: 0.07, 0.41)
Female Holstein	200.1 ± 39.9 (N: 8.8, 357)	Fixed cost lactating cows, \$/cow per day	5.0 ± 0.25 (N: 3.9, 6.1)
Male Holstein	25.0 ± 5.0 (N: 3.3, 47.3)	Fixed cost dry cows, \$/cow per day	2.0 ± 0.2 (N: 3.9, 6.1)
Crossbred	180 ± 34.9 (N: 27.7, 315.6)	Replacement cost,[3] \$/heifer	1,485 ± 181 (N: 724, 2,247)
Primiparous offspring value, \$/calf	170 ± 35 (N: 25.8, 327.8)	Semen,[5] \$/dose	
		Sex-sorted Holstein	18 ± 3 (N: 4.2, 30.5)
		Beef	5.0 ± 0.5 (N: 2.9, 7.1)
		GnRH and $PGF_{2\alpha}$,[6] \$/dose	1.8 ± 0.2 (N: 0.7, 2.9)
		Human chorionic gonadotropin, \$/dose	10 ± 1 (N: 5.75, 14.5)
		AMD cost,[7] \$/cow per day	0.09 ± 0.01 (N: 0.04, 0.13)
		Labor cost[8]	
		Insemination, \$/AI	0.83 ± 0.12 (U: 0.63, 1.04)
		Reproductive hormone, \$/treatment	0.25 ± 0.03 (U: 0.19, 0.31)
		AMD fitting, \$/h	0.50 ± 0.07 (U: 0.38, 0.62)
		Reproductive exams, \$/exam	3.0 ± 0.6 (U: 2.0, 4.0)

[1]Dist. = distribution (N = normal, U = uniform), min.: minimum, max.: maximum.

[2]Source: USDA-NASS (2022a).

[3]Source: Historical Cattle Prices (Schulz, 2020; USDA-NASS, 2022b,c).

[4]Source: USDA-ERS (2023).

[5]Source: Olynk and Wolf (2007), and confirmed with 5 commercial herds in north-central Florida and south-central Georgia.

[6]Market price from Southeast Milk Inc. (Belleview, FL).

[7]Market price from Merck Animal Health (Rahway, NJ).

[8]Source: USDA-NASS (2022d,e).

follicles ≥10 mm in diameter were recorded. Cows that on the day of the GnRH treatment had follicles ≥10 mm and on the day of the $PGF_{2\alpha}$ treatment had a new CL ≥20 mm in diameter were considered to have had ovulation in response to the GnRH treatment. The same cows had blood (~7 mL) collected from the coccygeal vein or artery into evacuated tubes containing EDTA (10 mL; BD Vacutainer K2 EDTA Blood Collection Tube, Becton, Dickinson and Company, San Diego, CA) at the time of the first GnRH and $PGF_{2\alpha}$ injections of the Ovsynch protocol for analysis of progesterone concentration. Within 2 h of collection, samples were centrifuged (10 min, 2,000 × g at 15°C) and plasma was stored in 2-mL Eppendorf tubes at −20°C until analyzed. The progesterone assay was carried out using a validated immunoassay (Megahed et al., 2023). Briefly, the IMMULITE 2000 XPi automated closed system (Siemens Healthineers) was used to measure plasma progesterone. Commercially available quality control samples (Lyphochek Trilevel, Bio-Rad, Hercules, CA) were run daily in duplicate in accordance with the manufacturer's protocol. Additionally, male calf plasma was spiked with certified progesterone reference material (Cerilliant Sigma, St. Louis, MO) and run in duplicate daily as a positive control. The spiking concentrations were 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, and 5.0 ng/mL. The CV for all control samples was less than 10%.

Partial Budget Analysis

All calculations were made using Excel. Monetary values reported by the collaborating herds during the experiment for each of the variables included in the gross profit calculation are described in Table 1.

Gross profit was calculated from calving to the date the cow was sold, died, or initiated a new lactation, as follows:

$$\begin{aligned}\text{Gross profit} &= (\text{milk income} + \text{sale value} \\ &+ \text{subsequent lactation calf value}) \\ &- (\text{feed cost} + \text{replacement cost} + \text{fixed cost} \\ &+ \text{depreciation} + \text{reproductive management cost}).\end{aligned}$$

To assess the economic value of keeping a cow in the herd for an additional lactation compared with replacing her with a first lactation cow based on future profitability, we calculated the retention payoff (**RPO**; De Vries, 2006; Cabrera, 2012) as follows:

$$\begin{aligned}\text{RPO} &= \{[(\text{value of replacement animal} \\ &+ \text{average value of offspring of first lactation cows}) \\ &- \text{average sale value}_{\text{LactNum}}) \times (\text{maximum LactNum} \\ &- \text{LactNum prior to calving})]/\text{maximum LactNum}\},\end{aligned}$$

where LactNum = lactation number from 0 to 6. Subsequently, we calculated the adjusted gross profit to account for the RPO as follows:

$$\text{adjusted gross profit} = \text{gross profit} + \text{RPO}.$$

To calculate the estimated sale value of individual cows, we estimated BW on the day of sale using the formula by van Arendonk (1985) described subsequently. Female Holstein calves born from twin pregnancies with a male calf were considered freemartin and were given the value of a male Holstein calf. The average value of a first lactation cow's offspring was calculated based on the collaborating herds' use of sex-sorted Holstein (80%), conventional Holstein (10%), and conventional beef (10%) semen among nulliparous animals and the observed stillbirth rate of 7% among first lactation cows. Thus, the average value of a first lactation cow's offspring was estimated to be US$170.

Costs associated with reproductive management included the cost of reproductive hormones, AI, semen, and reproductive tract examinations and the cost of AMD for the TRM treatment. Based on observations carried out during the experiment in the collaborating herds, we estimated that the cost of supplies for treatment with reproductive hormones was US$0.10/treatment and that 60 cows could be treated per hour. The cost of supplies per AI was US$0.50 (sheath, sleeve, semen applicator, water bath), and 30 cows could be AI per hour.

Income from milk over feed cost (**IOFC**) during the lactation period was calculated as follows:

$$\text{IOFC} = \text{total milk revenue} - \text{total lactating cow feed cost}.$$

Estimating DMI and Live BW

Dry matter intake during lactation was calculated using the NRC (2021) guidelines, employing the following equation:

$$\text{DMI (kg/d)} = [3.7 + (\text{Parity} \times 5.7) + 0.305 \times \text{MilkE (Mcal/d)} + 0.022 \times \text{BW (kg)} + (-0.689 - 1.87 \times \text{Parity}) \times \text{BCS}] \times [1 - (0.212 + \text{Parity} \times 0.136) \times e^{(-0.053 \times \text{DIM})}],$$

where MilkE (milk energy) = (0.0929 × %Fat × yield) + (0.0547 × %True Protein × yield) + (0.0395 × %Lactose × yield). Milk component data were unavailable. Thus, the average milk component from average bulk tank milk composition by month were used to estimate MilkE.

Body condition score was assumed to remain constant at 3.25 (Ferguson et al., 1994). Dry matter intake during the dry period was estimated as 1.8 kg/100 kg of BW, assuming a dietary NDF content of ~50% (NRC, 2021). Body weight was estimated based on the following equation from van Arendonk (1985):

$$BW_{tatltp} = f(\text{age}) + f(\text{lactation}) + f(\text{pregnancy}) = A\{1 - [1 - (y_0 A^{-1})^{1/3}] \exp(-kt_a)\}^3 + p_1 t_l p_2^{-1} \exp(1 - t_l p_2^{-1}) + p_3^3 t_{pc}^3,$$

where BW_{tatitp} = liveweight of a cow t_a days old at t_l days in lactation and t_p days pregnant; t_a = age (days); t_l = number of days in lactation; t_p = number of days after conception; A = mature liveweight (kg); y_0 = birth weight (kg); k = growth rate parameter; p_1 = maximum decrease of liveweight during the lactation (kg); p_2 = time during the lactation with the minimum liveweight (days); p_3 = pregnancy parameter; $t_{pc} = t_p - 50$ when $t_p - 50 > 0$, otherwise $t_{pc} = 0$.

Based on herd data, mature BW and birth BW were set at 690 kg and 42 kg, respectively, with a growth rate parameter (k) of 0.0028. Maximum BW loss during lactation (p_1) was 30 kg for primiparous cows and 50 kg for multiparous cows. The time to minimum BW (p_2) was assumed to be 60 d for primiparous and 80 d for multiparous cows.

Stochastic Analysis

A stochastic Monte Carlo simulation model was developed using SAS (SAS/SAT version 9.4, SAS Institute Inc., Cary, NC) to estimate differences in gross profit between the control and TRM treatments when values for inputs and outputs vary (Table 2). Fixed inputs were calculated for each treatment to determine differences in gross profit and adjusted gross profit between treatments and for each treatment within parity (primiparous vs. multiparous) to determine differences in gross profit between treatments according to parity. Fixed inputs included average milk production, percentage of cows sold and average BW at sale, percentage of cows starting a new lactation, percentage of cows culled (sold or died), number of live Holstein female and male calves and crossbred calves born, average DMI (lactating + dry periods), average days in the herd (DIM + days dry), number of doses of semen (Holstein and beef conventional, Holstein sex-sorted), number of AI, number of reproductive tract examinations, and number of treatments with reproductive hormones.

Stochasticity in every iteration of the simulation was introduced for milk price, cow sale price, value of 1-d-old calves (Holstein female and male, crossbred), feed cost, fixed cost, replacement cost, semen cost, labor cost (AI, reproductive tract examination, reproductive hormone treatment, fitting AMD), daily cost of AMD. Historical prices (i.e., January 2012 to October 2021) of milk (USDA-NASS, 2022a), cow sales (Schulz, 2020; USDA-NASS, 2022b), and replacement cost (USDA-NASS, 2022c) and feed cost (USDA-ERS, 2023) were retrieved from USDA electronic databases. Labor cost for performing hormonal treatments, fitting collars, and AI were gathered from USDA (USDA-NASS, 2022d,e) and from the collaborating herds and 10 other dairies from across Florida and Georgia (personal communication, Ricardo C. Chebel, Gainesville, FL). Semen cost was based on what was reported for the collaborating herds and 10 other dairies from across Florida and Georgia (personal communication, Ricardo C. Chebel, Gainesville, FL).

Simulations were run and recorded for 100,000 iterations. For each iteration, the gross profit and adjusted gross profit were calculated for each treatment and parity as follows:

$$
\begin{aligned}
\text{Gross profit} = {} & [(\text{yield} \times \text{milk price}) \\
& + (\%\ \text{cows sold} \times \text{estimated BW} \times \text{cow sale value}) \\
& + (\text{Holstein female calves} \times \text{calf value}) \\
& + (\text{Holstein male calves} \times \text{calf value}) \\
& + (\text{crossbred calves} \times \text{calf value})] \\
& - [(\text{DMI} \times \text{lactating cow feed cost}) \\
& + (\text{DMI} \times \text{dry cow feed cost}) \\
& + (\text{days in the herd} \times \text{fixed cost}) \\
& + (\%\ \text{cows replaced} \times \text{replacement cost}) \\
& + (\text{days in the herd} \times \text{AMD daily cost}) \\
& + (\text{sex-sorted Holstein semen doses} \times \text{semen cost}) \\
& + (\text{Holstein semen doses} \times \text{semen cost}) \\
& + (\text{beef semen doses} \times \text{semen cost}) \\
& + (\text{number of AI} \times \text{cost of AI supplies}) \\
& + (\text{number of AI} \times \text{labor cost}) + (\text{reproductive} \\
& \quad \text{examinations} \times \text{labor cost}) \\
& + (\text{reproductive hormone treatments} \times \text{cost of hormone}) \\
& + (\text{reproductive hormone treatments} \times \text{labor cost})], \text{ and}
\end{aligned}
$$

$$
\begin{aligned}
\text{Adjusted gross profit} = {} & \text{gross profit} \\
& + (\%\ \text{cows}_{\text{LactNum}} \times \%\ \text{cows replaced} \times \text{RPO}) \\
& + (\%\ \text{cows}_{\text{LactNum}} \times \%\ \text{cows retained} \times \text{RPO}).
\end{aligned}
$$

Statistical Analysis

This was a completely randomized, block clinical trial, with cows blocked according to parity (primiparous vs. multiparous). The experiment was designed as a noninferiority trial to determine whether the likelihood of pregnancy at 67 d after the first AI would not differ between the control and TRM treatments. As such, we determined that while protecting against type I ($\alpha = 0.10$) error, 1,300 cows per treatment would be required when the true difference in pregnancy at 67 d after the first AI in favor of the control treatment is 5% (e.g., control = 55%, TRM = 50%) to ensure that the probability of type II error is 10% ($1 - \beta = 0.90$). In addition, to compare the pregnancy outcomes of anestrus cows presynchronized with the Ovsynch versus hCG, we determined that a total of 1,500 anestrus cows would be needed (750/treatment) to protect against type I ($\alpha = 0.10$) and II ($1 - \beta = 0.90$) errors when the true difference in pregnancy at 67 d after the first AI in favor of the control treatment is 6.5% (e.g., control = 45%, TRM = 38.5%). The cows used for progesterone concentration and ovulation analysis were a convenience sample. A post hoc sample size calculation revealed that the sample size used was sufficient ($\alpha = 0.05$, $1 - \beta = 0.80$) to detect differences in progesterone concentration of 1.2 ng/mL when the SD is 2.7 ng/mL. Similarly, a post hoc sample size calculation revealed that the sample size used was sufficient ($\alpha = 0.05$, $1 - \beta = 0.80$) to detect a difference in probability of ovulation of 0.20 when the probabilities of ovulation of the control and TRM1 treatments are 0.70 and 0.50, respectively.

Statistical analyses were carried out with using SAS. Descriptive data at the time of enrollment were analyzed by chi-squared and Fisher's exact test using the FREQ procedure (e.g., percentage of primiparous cows, percentage of cows calving males, incidence of calving disorders, incidence of postpartum diseases) and univariable ANOVA using the MIXED procedure (e.g., genomic estimated breeding values, BW at calving, gestation length, days dry, days on the close-up diet).

Continuous outcomes were analyzed by ANOVA using the MIXED procedure. Normality was assessed by visual inspection of distribution of residuals and variables that did not have normal distribution were transformed using log natural or square root function. In those cases, responses are presented as back-transformed values. When transformation of continuous variables did not result in normal distribution of residuals (e.g., cow sales, calf value, dry cow feed cost, replacement cost), we used the Kruskal–Wallis test (NPAR1WAY procedure) with treatment or parity as univariable fixed effects. Ordinal outcomes (e.g., number of AI, treatments with reproductive hormones, reproductive examinations, and live calves in the subsequent lactation) were analyzed using negative binomial regression (GENMOD procedure). We analyzed binomial outcomes by logistic regression using the LOGISTIC procedure. Results referent to the logistic regression are presented as the adjusted OR and LSM and corresponding 95% CI. Least squares means were calculated using the logit link function. Hazard of pregnancy and hazard of initiating a new lactation were analyzed by Cox proportional hazard regression using the PHREG procedure and results are presented as the adjusted hazard ratio and corresponding 95% CI. In logistic and Cox proportional hazard regressions, the control treatment was always set as referent. Chi-squared was used to analyze the percentage of anestrus cows with CL ≥20 mm at the time of the first GnRH and $PGF_{2α}$ injections of the Ovsynch protocol and the percentage of cows with presumptive ovulation between the first GnRH and $PGF_{2α}$ injections of the Ovsynch protocol. Univariable ANOVA was used to analyze the effect of treatment on the concentrations of progesterone on the day of the first GnRH and $PGF_{2α}$ injections of the Ovsynch protocol.

The multivariable models included treatment, parity (primiparous vs. multiparous) and the interaction between treatment and parity, EPEC (anestrus vs. estrual) and the interaction between treatment and EPEC, and number of days cows were exposed to THI ≥68 relative to calving (lactation performance) or AI (pregnancy outcomes). When analyzing P/AI, we also included in the models type of AI (estrus vs. TAI) and semen (Holstein sex-sorted semen, Limousin, Angus). Because of the differences in reproductive management of anestrus and estrual cows, we analyzed pregnancy outcomes to the first postpartum AI for all cows comparing the control and TRM (TRM1 + TRM2 + TRM3) treatments; for anestrus cows comparing the control and TRM1 treatments; and for estrual cows comparing the control, TRM2, and TRM3 treatments (control vs. TRM2 + TRM3, TRM2 vs. TRM3). In the analyses of the data of anestrus and estrual cows, we did not include EPEC in the models. To evaluate the interaction between treatment and gDPR in the 2,360 (89.6%) cows with pregnancy outcomes and gDPR, we included in the model quartiles of gDPR and the interaction between quartile of gDPR and treatment. Treatment was forced in all models, but independent variables with $P > 0.10$ were excluded from the model until only variables with $P \leq 0.10$ remained.

Statistical significance was declared at $P \leq 0.05$ and tendencies at $0.05 < P \leq 0.10$.

RESULTS

Descriptive Statistics

From the 3,482 cows that calved between August and October 2022, 15% were excluded before 45 ± 3 DIM, 0.9% were excluded between enrollment and first postpartum AI, and 8.2% were excluded after the first AI. Reasons for exclusion are depicted in Table 3. The final dataset included 2,635 cows.

The percentage of primiparous cows in the control (35.0%) and TRM (36.3%) treatment did not ($P = 0.47$) differ. Genomic estimated breeding values for net merit (control = US$344.3 ± 4.6, TRM = US$345.3 ± 4.8), milk (control = 160.6 ± 15.8, TRM = 178.2 ± 16.5 kg), gDPR (control = 0.23 ± 0.04, TRM = 0.16 ± 0.04), and cow conception rate (control = 0.87 ± 0.04, TRM = 0.79 ± 0.04) were not ($P \geq 0.19$) different between treatments. The quartiles of gDPR were as follows: Q1 = −4.6 to −0.7, Q2 = −0.6 to 0.2, Q3 = 0.3 to 1.0, and Q4 = 1.1 to 3.8. Furthermore, BW at calving (primiparous: control = 660.8 ± 3.6, TRM = 659.0 ± 3.7 kg; multiparous: control = 744.0 ± 2.6, TRM = 743.2 ± 2.8 kg), gestation length (control = 277.9 ± 0.2, TRM = 277.9 ± 0.3 d), and days on the close-up diet (control = 27.0 ± 0.4, TRM = 26.6 ± 0.4 d) were not ($P \geq 0.49$) different between treatments. The length of the dry period was ($P = 0.05$) shorter for the control treatment (control = 60.1 ± 0.3, TRM = 60.8 ± 0.3 d). The percentage of cows that calved male calves (control = 31.0%, TRM = 32.7%) and had calving problems (control = 12.8%, TRM = 11.0%), retained fetal membranes (control = 2.3%, TRM = 2.4%), metritis (control = 11.2%, TRM = 12.0%), ketosis ≤21 DIM (control = 8.2%, TRM = 9.6%), indigestion ≤60 DIM (control = 11.1%, TRM = 12.7%), displaced abomasum ≤60 DIM (control = 1.8%, TRM = 2.1%), mastitis ≤60 DIM (control = 14.3%, TRM = 12.3%), and lameness ≤60 DIM (control = 0.07%, TRM = 0.00%) were not ($P \geq 0.13$) different between treatments. Milk yield in the first 28 DIM was not ($P = 0.86$) different between treatments (primiparous: control = 30.0 ± 0.3, TRM = 30.0 ± 0.3 kg/d; multiparous: control = 45.8 ± 0.2, TRM = 45.6 ± 0.3 kg/d). Multiparous cows in the control treatment tended ($P = 0.08$) to be more likely to have milk fever ≤21 DIM (control = 0.7%, TRM = 0.1%). Finally, we observed no ($P = 0.16$) difference between treatments in the likelihood of cows having an intense estrus by 45 ± 3 DIM (Table 4). The final dataset included 772 and 740 anestrus cows enrolled in the control and TRM treatments, respectively, and 600 and 523 estrual cows enrolled in the control and TRM treatments, respectively.

Effects of Treatment on First and Second Inseminations

Results referent to the first AI are presented in Table 4. Per experimental design, a greater ($P < 0.01$) percentage of control cows were enrolled in an OvSP. Among cows enrolled in the OvSP, those in the control treatment were ($P < 0.01$) more likely to finish the protocol and be inseminated at fixed time. Consequently, cows in the control treatment received ($P < 0.01$) more hormonal treatments before first AI. Treatment affected ($P \leq 0.05$) the percentage of cows pregnant at 32 (control = 56.7% [95% CI = 54.0%, 59.3%], TRM = 52.5% [95% CI = 49.7%, 55.3%]) and 67 (control = 53.9% [95% CI = 51.1%, 56.6%], TRM = 50.1% [95% CI = 47.2%, 53.0%]) d after the first AI, but there was only a tendency ($P = 0.07$) for a greater percentage of control cows to calve to the first AI (49.5% [95% CI = 46.7%, 52.3%] vs. 45.8% [95% CI = 42.9%, 48.7%]). Treatment did not ($P \geq 0.55$) affect pregnancy loss from 32 to 67 d of gestation (control = 4.2% [95% CI = 2.9%, 5.9%], TRM = 4.1% [95% CI = 2.8%, 5.9%]) and from 67 d of gestation to calving (control = 4.7% [95% CI = 3.3%, 6.7%], TRM = 4.1% [95% CI = 2.7%, 6.1%]). The interactions between treatment and parity ($P \geq 0.46$; Table 4) and between treatment and EPEC ($P \geq 0.15$; Figure 2) did not affect pregnancy outcomes following the first AI. The percentage of cows pregnant at any given time after the first AI was ($P < 0.01$) ~16% lower for anestrus than estrual cows (32 d: anestrus = 49.8% [95% CI = 47.3%, 52.3%], estrual = 59.3% [95% CI = 56.4%, 62.1%]; 67 d: anestrus = 47.6% [95% CI = 45.0%, 50.1%], estrual = 56.4% [95% CI = 53.3%, 59.4%]; calving: anestrus = 43.7% [95% CI = 41.1%, 46.4%], estrual = 51.6% [95% CI = 48.4%, 54.7%]), but EPEC was not ($P \geq 0.13$) associated with pregnancy loss at any time point. The interaction between treatment and gDPR did not ($P \geq 0.18$) affect pregnancy outcomes to the first AI (Supplemental Figures S1 and S2, see Notes). As expected, however, the highest quartiles of gDPR had the highest P/AI ($P \leq 0.01$), but gDPR was not ($P \geq 0.43$) associated with pregnancy loss.

Cows enrolled in the TRM treatment tended ($P = 0.06$) to have greater hazard of second AI (control = referent, TRM = 1.18 [95% CI = 0.99, 1.39]), but the likelihood of second AI at fixed time was not ($P = 0.41$) affected by treatment (control = referent, TRM = 0.91 [95% CI = 0.71, 1.15]). Treatment did not ($P \geq 0.41$) affect pregnancy outcomes after the second AI (pregnancy 32 d = 50.0%, pregnancy 67 d = 46.1%, pregnancy loss between 32 and 67 d of gestion = 7.3%, calving = 40.8%, pregnancy loss from 67 d of gestation to calving = 5.5%). Although EPEC did not ($P = 0.13$) affect P/AI 32 d after the second AI (anestrus = 49.5% [95% CI = 45.9%, 53.1%], estrual = 54.0% [95% CI = 49.2%, 58.8%]), because anestrus cows were ($P = 0.05$) more likely to lose a pregnancy from 32 to 67 d after the second AI (anestrus = 9.1% [95% CI = 6.5%, 12.5%], estrual = 4.7% [95% CI = 2.7%, 8.2%]), the former had ($P \leq 0.05$) lesser P/AI at 67 d (anestrus = 44.7% [95% CI = 41.1%, 48.3%], estrual = 50.7% [95% CI = 45.9%, 55.5%]) and to term (anestrus = 39.3% [95% CI = 35.7%, 43.0%], estrual = 46.7% [95% CI = 41.8%, 51.6%]) after the second AI.

Table 3. Distribution of cows according to reason for exclusion from the experiment

Reason for exclusion	Calving to enrollment (all cows)	Enrollment to first AI		After first AI	
		Control[1]	TRM[2]	Control[1]	TRM[2]
Not eligible for AI	383	3	4	5	2
Sold	95	7	12	126	119
Dead	51	2	0	5	8
Noncompliant	0	14	11	0	0
Total	529	14	17	148	139

[1]Control: cows subjected to the Double-Ovsynch protocol (Souza et al., 2008) at 46 ± 3 DIM for first postpartum AI at fixed time (86 μg of gonadorelin at 46 ± 3 DIM, 500 μg of cloprostenol sodium at 53 ± 3, 86 μg of gonadorelin at 56 ± 3 and 63 ± 3 DIM, 500 μg of cloprostenol sodium at 70 ± 3 and 71 ± 3 DIM, 86 μg of gonadorelin at 72 ± 3 DIM, fixed time AI at 73 ± 3 DIM). Cows were re-inseminated upon detection of estrus by an automated monitoring device (AMD).

[2]TRM: TRM1 = cows without a AMD-detected estrus with high heat index (primiparous ≥90, multiparous ≥70; 0 = minimum, 100 = maximum) by 45 ± 3 DIM were enrolled in the hCG-Ovsynch (2,000 IU of human chorionic gonadotropin at 56 ± 3 DIM and the Ovsynch protocol 7 d later [86 μg of gonadorelin at 63 ± 3 DIM, 500 μg of cloprostenol sodium at 70 ± 3 and 71 ± 3 DIM, 86 μg of gonadorelin at 72 ± 3 DIM, fixed time AI at 73 ± 3 DIM]). TRM2 = cows with at least one high heat index by 45 ± 3 DIM were monitored and received a $PGF_{2\alpha}$ treatment at 60 to 73 DIM, when they were 6 to 22 d after estrus. Cows were inseminated in estrus for 7 d and those not detected in estrus were enrolled in the hCG-Ovsynch, 67 to 80 DIM. TRM3 = cows that had had a high intensity estrus by 45 ± 3 DIM but where ≥23 d after estrus at 63 ± 3 DIM were enrolled in the hCG-Ovsynch. Cows were re-inseminated upon detection of estrus by the AMD.

Anestrus Cows: Effects of Treatment on Corpus Luteum, Progesterone Concentration, Presumptive Ovulation, and Pregnancy Outcomes

Although anestrus cows in the control treatment were ($P = 0.02$) more likely to have a CL on the day of the first GnRH injection of the Ovsynch protocol (Figure 3A), they had ($P < 0.01$) lower progesterone concentration than cows in the TRM1 treatment (Figure 3B). On the day of the first $PGF_{2\alpha}$ injection of the Ovsynch protocol, anestrus cows in the control treatment were ($P < 0.02$) more likely to have a CL and had ($P < 0.01$) greater progesterone concentration (Figure 3B). The percentage of anestrus cows with presumptive ovulation between the first GnRH and first $PGF_{2\alpha}$ injections of the Ovsynch protocol did not ($P = 0.14$) differ between treatments (Figure 3A). Among anestrus cows, we did not ($P \geq 0.11$) detect an effect of treatment on P/AI at 32 and 67 d of gestation and to term (Figure 2A).

Estrual Cows: Effects of Treatment on Pregnancy Outcomes

Among estrual cows enrolled in the TRM treatment, 62.3% (n = 326) were treated with $PGF_{2\alpha}$ between 60 and

Table 4. Effect of treatment on first postpartum insemination outcomes

Item	Primiparous		Multiparous		*P*-value		
	Control[1] (n = 480)	TRM[2] (n = 459)	Control[1] (n = 892)	TRM[2] (n = 804)	TRT	Parity	TRT × parity
Cows with intense estrus by 45 ± 3 DIM					0.16	<0.01	0.16
Adjusted OR (95% CI)	Referent	0.78 (0.59, 1.02)	Referent	0.99 (0.82, 1.20)			
LSM, % (95% CI)	37.3 (33.1, 41.7)	31.6 (27.5, 36.0)	47.2 (43.9, 50.5)	47.0 (43.6, 50.5)			
Fist postpartum AI							
Cows enrolled in an OvSP, %	100.0	81.9	100.0	82.3	<0.01	0.68	—
Cows enrolled in OvSP inseminated at TAI					<0.01	<0.01	0.90
Adjusted OR (95% CI)	Referent	0.39 (0.19, 0.82)	Referent	0.39 (0.26, 0.60)			
LSM, % (95% CI)	97.5 (95.6, 98.6)	93.9 (90.6, 96.0)	95.6 (94.1, 96.8)	89.6 (86.9, 91.8)			
Reproductive hormone treatments, LSM ± SEM	6.98 ± 0.12	3.96 ± 0.09	6.96 ± 0.09	4.18 ± 0.07	<0.01	0.31	0.11
Days in milk at first AI,[3] LSM	73.0	72.3	72.9	72.9	0.03	<0.01	—
Pregnancy 32 d					0.03	0.11	0.98
Adjusted OR (95% CI)	Referent	0.84 (0.65, 1.09)	Referent	0.84 (0.69, 1.02)			
LSM, % (95% CI)	58.8 (54.0, 63.4)	54.5 (49.7, 59.3)	54.9 (51.4, 58.4)	50.6 (46.9, 54.2)			
Pregnancy 67 d					0.05	0.03	0.85
Adjusted OR (95% CI)	Referent	0.85 (0.66, 1.10)	Referent	0.86 (0.71, 1.05)			
LSM, % (95% CI)	56.7 (51.9, 61.4)	52.6 (47.7, 57.4)	51.1 (47.6, 54.6)	47.5 (43.9, 51.2)			
Pregnancy loss (32 to 67 d)					0.91	0.06	0.58
Adjusted OR (95% CI)	Referent	1.12 (0.42, 3.05)	Referent	0.92 (0.51, 1.66)			
LSM, % (95% CI)	3.0 (1.5, 5.9)	3.4 (1.7, 6.6)	5.5 (3.8, 8.0)	5.1 (3.4, 7.8)			
Calving					0.07	<0.01	0.81
Adjusted OR (95% CI)	Referent	0.85 (0.65, 1.10)	Referent	0.87 (0.71, 1.06)			
LSM, % (95% CI)	53.4 (48.5, 58.2)	49.3 (44.4, 54.2)	45.7 (42.2, 49.3)	42.3 (38.6, 46.0)			
Pregnancy loss (67 d to calving)					0.55	<0.01	0.46
Adjusted OR (95% CI)	Referent	1.31 (0.43, 3.96)	Referent	0.77 (0.44, 1.35)			
LSM, % (95% CI)	2.5 (1.1, 5.4)	3.2 (1.5, 6.6)	7.7 (5.5, 10.6)	6.0 (4.0, 9.1)			

[1]Control: cows subjected to the Double-Ovsynch protocol (Souza et al., 2008) at 46 ± 3 DIM for first postpartum AI at fixed time (86 µg of gonadorelin at 46 ± 3 DIM, 500 µg of cloprostenol sodium at 53 ± 3, 86 µg of gonadorelin at 56 ± 3 and 63 ± 3 DIM, 500 µg of cloprostenol sodium at 70 ± 3 and 71 ± 3 DIM, 86 µg of gonadorelin at 72 ± 3 DIM, fixed time AI at 73 ± 3 DIM). Cows were re-inseminated upon detection of estrus by an automated monitoring device (AMD).

[2]TRM: TRM1 = cows without a AMD-detected estrus with high heat index (primiparous ≥90, multiparous ≥70; 0 = minimum, 100 = maximum) by 45 ± 3 DIM were enrolled in the hCG-Ovsynch (2,000 IU of human chorionic gonadotropin at 56 ± 3 DIM and the Ovsynch protocol 7 d later [86 µg of gonadorelin at 63 ± 3 DIM, 500 µg of cloprostenol sodium at 70 ± 3 and 71 ± 3 DIM, 86 µg of gonadorelin at 72 ± 3 DIM, fixed-time AI at 73 ± 3 DIM]). TRM2 = cows with at least one high heat index by 45 ± 3 DIM were monitored and received a $PGF_{2\alpha}$ treatment at 60 to 73 DIM, when they were 6 to 22 d after estrus. Cows were inseminated in estrus for 7 d and those not detected in estrus were enrolled in the hCG-Ovsynch, 67 to 80 DIM. TRM3 = cows that had had a high intensity estrus by 45 ± 3 DIM but where ≥23 d after estrus at 63 ± 3 DIM were enrolled in the hCG-Ovsynch. Cows were re-inseminated upon detection of estrus by the AMD.

[3]Results referent to nonparametric analysis (Kruskal-Wallis).

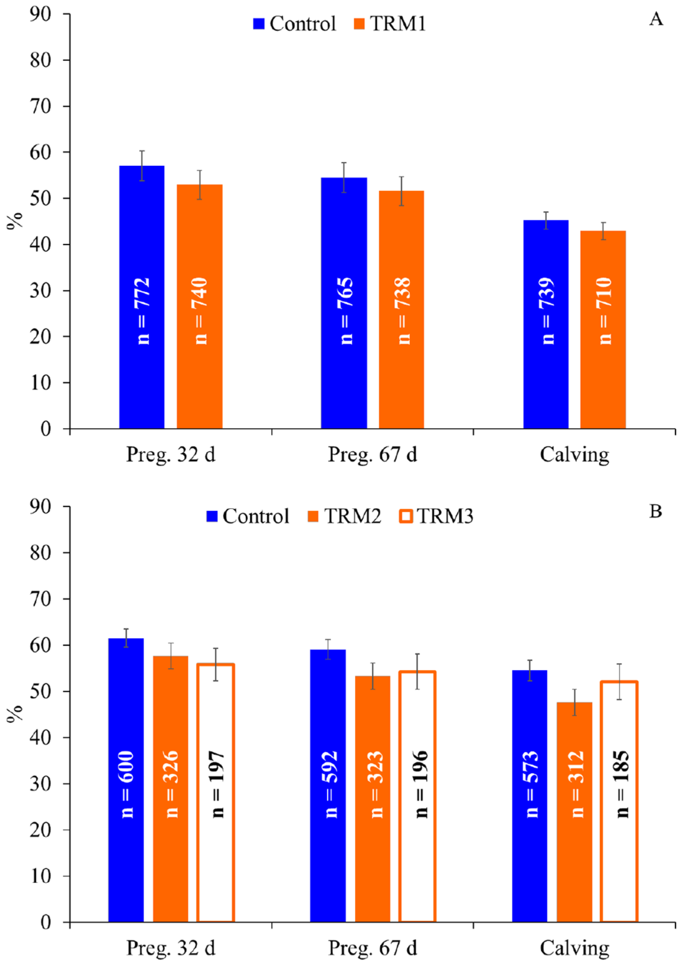

Figure 2. Effects of treatment and early postpartum estrus characteristics (EPEC) on pregnancy (preg.) outcomes to first postpartum AI. (A) Anestrus cows: cows without automated monitoring device (AMD) detected high intensity estrus (primiparous ≥90, multiparous ≥70; 0 = minimum, 100 = maximum) by 45 ± 3 DIM. (B) Estrual cows: cows with at least one AMD-detected high intensity estrus (primiparous ≥90, multiparous ≥70; 0 = minimum, 100 = maximum) by 45 ± 3 DIM. Control: Cows subjected to the Double-Ovsynch protocol (Souza et al., 2008) at 46 ± 3 DIM for first postpartum AI at fixed time (86 µg of gonadorelin at 46 ± 3 DIM, 500 µg of cloprostenol sodium at 53 ± 3, 86 µg of gonadorelin at 56 ± 3 and 63 ± 3 DIM, 500 µg of cloprostenol sodium at 70 ± 3 and 71 ± 3 DIM, 86 µg of gonadorelin at 72 ± 3 DIM, fixed-time AI at 73 ± 3 DIM). TRM: TRM1 = cows without an AMD-detected estrus with high heat index (primiparous ≥90, multiparous ≥70; 0 = minimum, 100 = maximum) by 45 ± 3 DIM were enrolled in the hCG-Ovsynch (2,000 IU of human chorionic gonadotropin at 56 ± 3 DIM and the Ovsynch protocol 7 d later [86 µg of gonadorelin at 63 ± 3 DIM, 500 µg of cloprostenol sodium at 70 ± 3 and 71 ± 3 DIM, 86 µg of gonadorelin at 72 ± 3 DIM, fixed-time AI at 73 ± 3 DIM]). TRM2 = cows with at least one high heat index by 45 ± 3 DIM were monitored and received a $PGF_{2\alpha}$ treatment at 60 to 73 DIM, when they were 6 to 22 d after estrus. Cows were inseminated in estrus for 7 d and those not detected in estrus were enrolled in the hCG-Ovsynch, 67 to 80 DIM. TRM3 = cows that had had a high intensity estrus by 45 ± 3 DIM but where ≥23 d after estrus at 63 ± 3 DIM were enrolled in the hCG-Ovsynch. All cows were re-inseminated upon detection of estrus by an AMD. Error bars represent SEM.

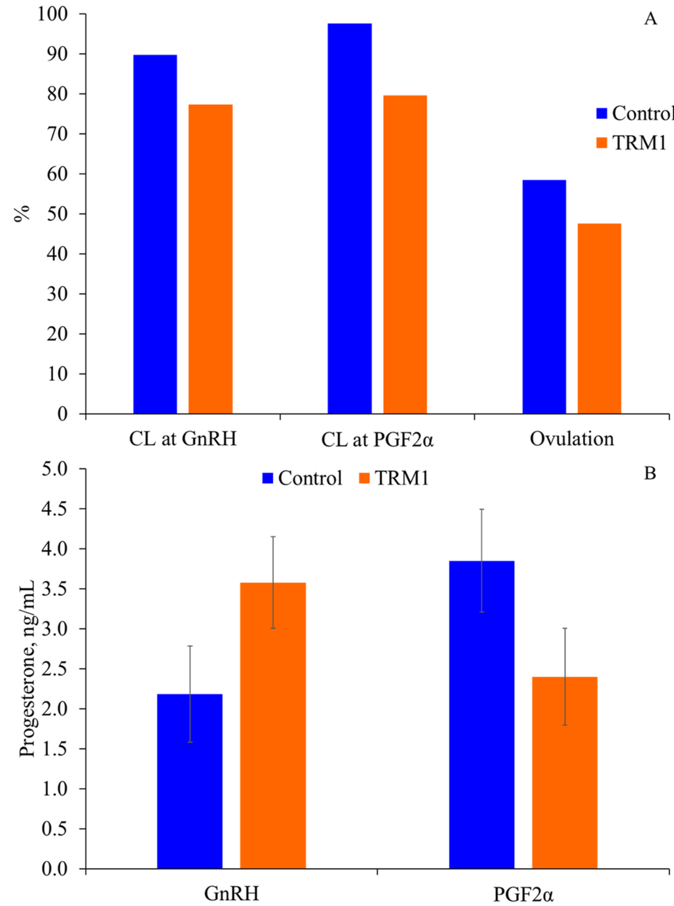

Figure 3. Effects of treatments on ovarian responses (A) and circulating progesterone concentration (B) of anovular cows during the Ovsynch protocol. Anestrus cows: cows without automated monitoring device (AMD) detected high intensity estrus (primiparous ≥90, multiparous ≥70; 0 = minimum, 100 = maximum) by 45 ± 3 DIM. Control: cows subjected to the Double-Ovsynch protocol (Souza et al., 2008) at 46 ± 3 DIM for first postpartum AI at fixed time (86 µg of gonadorelin at 46 ± 3 DIM, 500 µg of cloprostenol sodium at 53 ± 3, 86 µg of gonadorelin at 56 ± 3 and 63 ± 3 DIM, 500 µg of cloprostenol sodium at 70 ± 3 and 71 ± 3 DIM, 86 µg of gonadorelin at 72 ± 3 DIM, fixed-time AI at 73 ± 3 DIM). TRM1 = cows were enrolled in the hCG-Ovsynch at 56 ± 3 DIM (2,000 IU of human chorionic gonadotropin and the Ovsynch protocol 7 d later [86 µg of gonadorelin at 63 ± 3 DIM, 500 µg of cloprostenol sodium at 70 ± 3 and 71 ± 3 DIM, 86 µg of gonadorelin at 72 ± 3 DIM, fixed-time AI at 73 ± 3 DIM]). Effect of treatment on CL at GnRH: $P = 0.02$; CL at $PGF_{2\alpha}$: $P < 0.01$; presumptive ovulation between GnRH and $PGF_{2\alpha}$: $P = 0.14$; progesterone concentration at GnRH: $P < 0.01$; progesterone concentration at $PGF_{2\alpha}$: $P < 0.01$. Error bars represent SEM.

73 DIM and the remaining cows (n = 197) were subjected to the hCG-Ovsynch at 63 ± 3 DIM. Among estrual cows treated with $PGF_{2\alpha}$, 31% (n = 101) were not detected in estrus within 7 d of treatment and were subjected to the hCG-Ovsynch at 67 to 80 DIM. Among estrual cows enrolled in the OvSP, cows in the TRM treatment were ($P < 0.01$) less likely to be inseminated at fixed time at the end of the protocol (control = referent, TRM2 = 0.25 [95% CI

= 0.11, 0.57], TRM3 = 0.26 [95% CI = 0.14, 0.49]). The percentages of cows enrolled in the control, TRM2, and TRM3 treatments inseminated at a fixed time were 96.9% (95% CI = 95.2%, 98.0%), 25.7% (95% CI = 21.0%, 31.0%), and 86.5% (95% CI = 80.4%, 90.9%), respectively. It is worth noting that we did not ($P \geq 0.30$) detect effects of the interaction between treatment and type of AI on pregnancy outcomes of estrual cows. Furthermore, we did not ($P \geq 0.26$) detect an effect of the type of AI on percentage of cows pregnant at 32 d (TAI = 60.8% [95% CI = 57.4%, 64.0%], estrus = 55.3% [95% CI = 49.5%, 61.0%]) and 67 d (TAI = 57.5% [95% CI = 54.1%, 60.8%], estrus = 51.9% [95% CI = 46.1%, 57.7%]) and calving (TAI = 53.3% [95% CI = 49.5%, 57.1%], estrus = 47.3% [95% CI = 42.4%, 54.4%]) after the first AI.

Effect of Treatments on Lactation Performance

Zootechnical data regarding lactation performance are depicted in Table 5. Treatment did not ($P \geq 0.21$) affect the number of AI cows received and reproductive exams cows were subjected to. The number of reproductive hormone treatments cows received was affected ($P < 0.01$) by the interaction between treatment and EPEC because anestrus cows in the control treatment received 1.95 more treatments than TRM cows, whereas estrual cows in the control treatment received 3.64 more treatments than TRM cows. The interaction between treatment and EPEC tended ($P = 0.09$) to affect the hazard of pregnancy. Among anestrus cows, the hazard of pregnancy did not differ (control = referent, TRM = 0.98 [95% CI = 0.88, 1.09]), but estrual cows in the TRM treatment (0.85 [95% CI = 0.75, 0.96]) had a lower hazard of pregnancy than those in the control (referent). The likelihood of cows being deemed not eligible for AI, starting a new lactation, dying in the herd, and being sold was not ($P \geq 0.34$) affected by treatment. The estimated BW at sale was ($P = 0.03$) slightly lower for the TRM treatment. Treatment did not ($P \geq 0.48$) affect DIM, milk yield, and estimated intake during the lactation. Gestation length and estimated BW at dry-off, dry period length and estimated DMI, calving interval, and the number of live offspring produced were not ($P \geq 0.11$) affected by treatment.

Association Between Early Postpartum Estrus Characteristics and Lactation Performance

Anestrus cows received ($P < 0.01$), on average, 0.21 and 0.29 more AI and reproductive examinations, respectively, than estrual cows. As explained previously, the interaction between treatment and EPEC tended ($P = 0.09$) to affect the hazard of pregnancy. Among cows in the control treatment, the adjusted hazard of pregnancy of anestrus cows was 0.78 (95% CI = 0.70, 0.87; estrual = referent). The adjusted hazard of pregnancy of anestrus cows in the TRM treatment was 0.90 (95% CI = 0.80, 1.01; estrual = referent). Anestrus cows were ($P \leq 0.03$) more likely to be deemed not eligible for AI (6.5% [95% CI = 5.3%, 7.8%] vs. 4.5% [95% CI = 3.4%, 5.9%]), were less likely to start a new lactation (80.1% [95% CI = 77.9%, 82.1%] vs. 85.0% [95% CI = 82.8%, 87.1%]), and were more likely to be sold (18.3% [95% CI = 16.4%, 20.4%] vs. 13.7% [95% CI = 11.8%, 15.9%]) than estrual cows. Although DIM was not ($P = 0.11$) associated with EPEC, anestrus cows had ($P = 0.05$) greater milk yield (12,661.0 ± 77.8 kg vs. 12,423.0 ± 93.0 kg) and tended ($P = 0.10$) to have greater lactation estimated intake (7,104.7 ± 40.0 kg vs. 7,005.1 ± 47.8 kg) than estrual cows. Because anestrus cows tended ($P = 0.10$) to have greater estimated BW at dry-off than estrual cows (698.6 ± 2.0 kg vs. 693.6 ± 2.4 kg) and had ($P = 0.02$) longer dry period (56.2 ± 0.3 d vs. 55.2 ± 0.3 d), they had ($P = 0.01$) greater estimated intake during the dry period (708.9 ± 4.4 kg vs. 691.9 ± 5.2 kg) than estrual cows. Finally, the calving interval of anestrus cows was ($P < 0.01$) ~7 d longer than estrual cows (378.5 ± 1.2 d vs. 371.3 ± 1.4 d).

Partial Budget Analysis and Stochastic Analyses

The effects of treatment and EPEC on economic outcomes are depicted in Table 6. We did not ($P \geq 0.17$) detect differences between treatments regarding milk revenue, income from cow sales, and the value of calves born in the subsequent lactation. Furthermore, treatment did not ($P \geq 0.51$) affect feeding cost of lactating cows, feeding cost of dry cows, replacement costs, and fixed cost. The interaction between treatment and EPEC affected ($P < 0.01$) reproductive management costs. Among anestrus cows, the cost of reproductive management was greater for the TRM treatment but, among estrual cows, the cost of reproductive management was greater for the control treatment. We did not ($P \geq 0.21$) detect differences between treatments regarding IOFC, gross profit, retention payoff, and adjusted gross profit.

Anestrus cows had ($P = 0.05$) greater milk revenue than estrual cows ($6,001.2 ± $36.9 vs. $5,888.4 ± $44.1) and only tended ($P = 0.10$) to have greater lactating cow feed cost ($2,271.2 ± $12.8 vs. $2,239.3 ± $15.3). Consequently, IOFC was ($P = 0.04$) greater for anestrus than estrual cows ($3,730.0 ± 25.2 vs. $3,649.1 ± 30.1). Income from cow sales was ($P = 0.01$) greater for anestrus than estrual cows ($532.8 vs. $430.0), but the former had ($P < 0.01$) lower calf value ($242.3 vs. $264.3) and greater replacement cost ($423.3 vs. $336.6). We observed a tendency ($P = 0.10$) for anestrus cows to have lower dry cow feed cost than estrual cows ($150.2 ± $2.2 vs. $155.7 ± $2.6). Ultimately, EPEC was not ($P \geq 0.13$) associated with gross profit, retention payoff, and adjusted gross profit.

Table 5. Effects of treatments on lactational and reproductive performances according to parity

Item	Anestrus[1]		Estrual[2]		P-value		
	Control[3] (n = 772)	TRM[4] (n = 740)	Control[3] (n = 600)	TRM[4] (n = 523)	TRT	EPEC	TRT × EPEC
Artificial inseminations (± SEM)	2.00 ± 0.05	2.06 ± 0.05	1.79 ± 0.06	1.85 ± 0.06	0.21	<0.01	0.89
Reproductive hormone treatments (± SEM)	8.93 ± 0.13	6.98 ± 0.11	7.98 ± 0.14	4.34 ± 0.10	<0.01	<0.01	<0.01
Reproductive examinations (± SEM)	2.69 ± 0.06	2.71 ± 0.06	2.43 ± 0.07	2.40 ± 0.07	0.83	<0.01	0.65
Adjusted hazard of pregnancy (95% CI)	Ref.	0.98 (0.88, 1.09)	Ref.	0.85 (0.75, 0.96)	<0.01	<0.01	0.09
Cows deemed not eligible for AI, % (95% CI)	7.0 (5.4, 9.0)	6.4 (4.8, 8.4)	4.0 (2.7, 5.9)	5.7 (4.0, 8.1)	0.67	0.03	0.18
Cows starting a new lactation, % (95% CI)	79.6 (76.6, 82.3)	80.5 (77.5, 83.2)	86.2 (83.2, 88.7)	83.7 (80.3, 86.6)	0.68	<0.01	0.30
Cows dead in the herd, % (95% CI)	1.8 (1.1, 3.1)	1.0 (0.5, 2.0)	1.0 (0.5, 2.0)	1.1 (0.6, 2.4)	0.34	0.29	0.23
Cows sold, % (95% CI)	18.7 (16.1, 21.7)	17.9 (15.2, 20.9)	13.3 (10.7, 16.3)	14.2 (11.4, 17.6)	0.94	<0.01	0.53
Estimated BW at sale, kg (± SEM)	623.0 ± 8.2	603.5 ± 8.7	612.8 ± 11.0	592.0 ± 11.3	0.03	0.24	0.94
Days in milk (± SEM)	309.7 ± 2.2	312.0 ± 2.2	306.6 ± 2.5	307.3 ± 2.7	0.48	0.11	0.75
Lactation milk yield, kg (± SEM)	12,638.0 ± 109.4	12,687.0 ± 110.9	12,408.0 ± 127.1	12,431.0 ± 136.7	0.71	0.05	0.91
Lactation estimated intake, kg (± SEM)	7,077.1 ± 55.8	7,132.7 ± 56.7	6,999.3 ± 64.1	7,008.8 ± 68.7	0.55	0.10	0.70
Gestation length at dry-off, d (± SEM)	221.6 ± 0.5	222.0 ± 0.5	222.6 ± 0.5	221.9 ± 0.6	0.82	0.39	0.33
Estimated BW at dry-off, kg (± SEM)	698.8 ± 2.9	698.5 ± 2.9	694.0 ± 3.2	693.4 ± 3.5	0.97	0.10	0.97
Dry period length, d (± SEM)	56.2 ± 0.4	55.6 ± 0.4	55.4 ± 0.4	55.3 ± 0.5	0.55	0.02	0.57
Dry period estimated intake, kg (± SEM)	712.4 ± 6.2	705.7 ± 6.2	692.4 ± 7.0	691.7 ± 7.6	0.73	0.01	0.65
Calving interval, d (± SEM)	377.5 ± 1.6	379.4 ± 1.6	369.8 ± 1.8	372.9 ± 2.0	0.11	<0.01	0.74
Number of live calves	0.99 ± 0.04	1.02 ± 0.04	0.99 ± 0.05	1.03 ± 0.05	0.51	0.89	0.91

[1]Cows without AMD-detected estrus with high heat index (primiparous ≥90, multiparous ≥70; 0 = minimum, 100 = maximum) by 45 ± 3 DIM.

[2]Cows with at least one AMD-detected estrus with high heat index (primiparous ≥90, multiparous ≥70; 0 = minimum, 100 = maximum) by 45 ± 3 DIM.

[3]Control: cows subjected to the Double-Ovsynch protocol (Souza et al., 2008) at 46 ± 3 DIM for first postpartum AI at fixed time (86 μg of gonadorelin at 46 ± 3 DIM, 500 μg of cloprostenol sodium at 53 ± 3, 86 μg of gonadorelin at 56 ± 3 and 63 ± 3 DIM, 500 μg of cloprostenol sodium at 70 ± 3 and 71 ± 3 DIM, 86 μg of gonadorelin at 72 ± 3 DIM, fixed-time AI at 73 ± 3 DIM). Cows were re-inseminated upon detection of estrus by an automated monitoring device (AMD).

[4]TRM: TRM1 = cows without a AMD-detected estrus with high heat index (primiparous ≥90, multiparous ≥70; 0 = minimum, 100 = maximum) by 45 ± 3 DIM were enrolled in the hCG-Ovsynch (2,000 IU of human chorionic gonadotropin at 56 ± 3 DIM and the Ovsynch protocol 7 d later [86 μg of gonadorelin at 63 ± 3 DIM, 500 μg of cloprostenol sodium at 70 ± 3 and 71 ± 3 DIM, 86 μg of gonadorelin at 72 ± 3 DIM, fixed-time AI at 73 ± 3 DIM]). TRM2 = cows with at least one high heat index by 45 ± 3 DIM were monitored and received a $PGF_{2\alpha}$ treatment at 60 to 73 DIM, when they were 6 to 22 d after estrus. Cows were inseminated in estrus for 7 d and those not detected in estrus were enrolled in the hCG-Ovsynch, 67 to 80 DIM. TRM3 = cows that had had a high intensity estrus by 45 ± 3 DIM but where ≥23 d after estrus at 63 ± 3 DIM were enrolled in the hCG-Ovsynch. Cows were re-inseminated upon detection of estrus by the AMD.

Table 6. Effects of treatments on economic outcomes and gross profit according to parity

Item	Anestrus[1]		Estrual[2]		*P*-value		
	Control[3]	TRM[4]	Control[3]	TRM[4]	TRT	EPEC	TRT × EPEC
Output							
Milk revenue, $/cow	5,990.4 ± 51.8	6,013.7 ± 52.6	5,881.4 ± 60.3	5,892.1 ± 64.8	0.71	0.05	0.83
Cow sales,[5] $/cow	536.0	529.4	395.2	470.0	0.46	0.01	—
Calf value,[5] $/cow	236.2	248.7	258.6	270.8	0.17	<0.01	—
Input							
Lactating cow feed cost, $/cow	2,262.3 ± 17.8	2,280.1 ± 18.1	2,237.5 ± 20.5	2,240.5 ± 22.0	0.55	0.10	0.70
Dry cow feed cost,[5] $/cow	150.0 ± 3.0	150.3 ± 3.1	157.4 ± 3.5	154.0 ± 3.8	0.51	0.10	0.57
Replacement cost,[5] $/cow	435.2	410.8	310.0	367.1	0.69	<0.01	—
Fixed cost, $/cow	1,174.7 ± 8.4	1,182.4 ± 8.5	1,168.9 ± 9.6	1,168.5 ± 10.3	0.63	0.27	0.66
Reproductive management cost, $/cow	77.6[a] ± 0.8	82.2[b] ± 0.8	73.6[a] ± 0.9	69.5[b] ± 0.9	0.99	<0.01	<0.01
IOFC,[6] $	3,728.1 ± 35.4	3,733.4 ± 35.9	3,644.5 ± 41.1	3,651.3 ± 44.2	0.80	0.04	0.99
Gross profit,[7] $/cow	2,206.0 ± 33.5	2,235.1 ± 34.1	2,183.2 ± 38.5	2,202.4 ± 41.3	0.49	0.45	0.89
Retention payoff,[5,8] $/cow	1,230.6	1,252.0	1,148.6	1,165.9	0.21	0.29	—
Adjusted gross profit,[9] $/cow	3,492.1 ± 32.1	3,523.0 ± 32.6	3,432.3 ± 37.3	3,473.1 ± 40.2	0.25	0.13	0.89

[1]Cows without AMD-detected estrus with high heat index (primiparous ≥90, multiparous ≥70; 0 = minimum, 100 = maximum) by 45 ± 3 DIM.

[2]Cows with at least one AMD-detected estrus with high heat index (primiparous ≥90, multiparous ≥70; 0 = minimum, 100 = maximum) by 45 ± 3 DIM.

[3]Control: cows subjected to the Double-Ovsynch protocol (Souza et al., 2008) at 46 ± 3 DIM for first postpartum AI at fixed time (86 µg of gonadorelin at 46 ± 3 DIM, 500 µg of cloprostenol sodium at 53 ± 3, 86 µg of gonadorelin at 56 ± 3 and 63 ± 3 DIM, 500 µg of cloprostenol sodium at 70 ± 3 and 71 ± 3 DIM, 86 µg of gonadorelin at 72 ± 3 DIM, fixed-time AI at 73 ± 3 DIM). Cows were re-inseminated upon detection of estrus by an automated monitoring device (AMD).

[4]TRM: TRM1 = cows without a AMD-detected estrus with high heat index (primiparous ≥90, multiparous ≥70; 0 = minimum, 100 = maximum) by 45 ± 3 DIM were enrolled in the hCG-Ovsynch (2,000 IU of human chorionic gonadotropin at 56 ± 3 DIM and the Ovsynch protocol 7 d later [86 µg of gonadorelin at 63 ± 3 DIM, 500 µg of cloprostenol sodium at 70 ± 3 and 71 ± 3 DIM, 86 µg of gonadorelin at 72 ± 3 DIM, fixed-time AI at 73 ± 3 DIM]). TRM2 = cows with at least one high heat index by 45 ± 3 DIM were monitored and received a $PGF_{2\alpha}$ treatment at 60 to 73 DIM, when they were 6 to 22 d after estrus. Cows were inseminated in estrus for 7 d and those not detected in estrus were enrolled in the hCG-Ovsynch, 67 to 80 DIM. TRM3 = cows that had had a high intensity estrus by 45 ± 3 DIM but where ≥23 d after estrus at 63 ± 3 DIM were enrolled in the hCG-Ovsynch. Cows were re-inseminated upon detection of estrus by the AMD.

[5]Results referent to nonparametric analysis (Kruskal-Wallis).

[6]Income over feed cost (IOFC) = (milk sales − feed cost).

[7]Gross profit = (milk income + sale value + subsequent lactation calf value) − (feed cost + replacement cost + fixed cost + depreciation + reproductive management cost).

[8]Retention payoff = {[(value of replacement animal + offspring) − average sale value$_{\mathrm{LactNum}}$) × (maximum LactNum − LactNum before calving)]/maximum LactNum}, where LactNum = lactation number from 0 to 6.

[9]Adjusted gross profit = gross-profit + retention payoff.

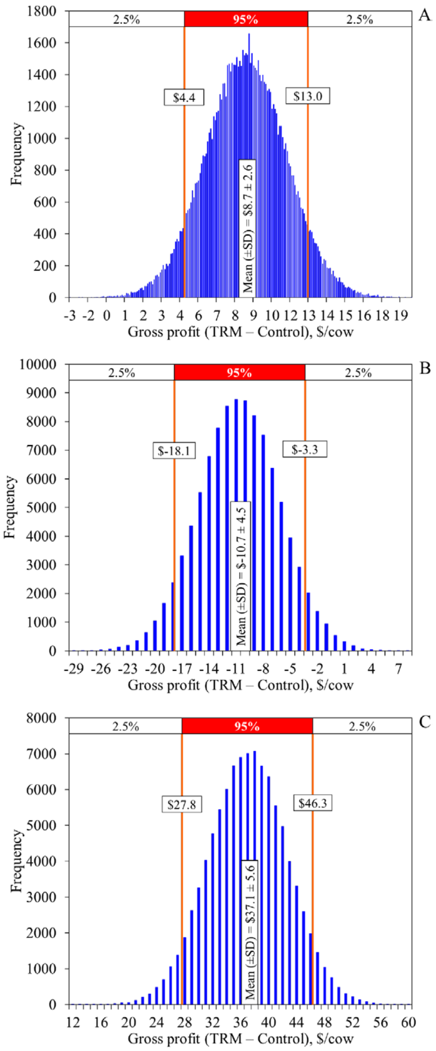

Figure 4. Relative frequency distribution for differences in gross profit between cows in the TRM and control treatments for 100,000 iterations of stochastic simulation. (A) Overall. (B) Anestrus cows: cows without automated monitoring device (AMD) detected high intensity estrus (primiparous ≥90, multiparous ≥70; 0 = minimum, 100 = maximum) by 45 ± 3 DIM. (C) Estrual cows: cows with at least one AMD-detected high intensity estrus (primiparous ≥90, multiparous ≥70; 0 = minimum, 100 = maximum) by 45 ± 3 DIM. Control: cows subjected to the Double-Ovsynch protocol (Souza et al., 2008) at 46 ± 3 DIM for first postpartum AI at fixed time (86 µg of gonadorelin at 46 ± 3 DIM, 500 µg of cloprostenol sodium at 53 ± 3, 86 µg of gonadorelin at 56 ± 3 and 63 ± 3 DIM, 500 µg of cloprostenol sodium at 70 ± 3 and 71 ± 3 DIM, 86 µg of gonadorelin at 72 ± 3 DIM, fixed-time AI at 73 ± 3 DIM). TRM: TRM1 = cows without an AMD-detected estrus with high heat index (primiparous ≥90, multiparous ≥70; 0 = minimum, 100 = maximum) by 45 ± 3 DIM were enrolled in the hCG-Ovsynch [2,000 IU of human chorionic gonadotropin at 56 ± 3 DIM and the Ovsynch protocol 7 d later (86 µg of gonadorelin at 63 ± 3 DIM, 500 µg of cloprostenol sodium at 70 ± 3 and 71 ± 3 DIM, 86 µg of gonadorelin at 72 ± 3 DIM, fixed-time AI at 73 ± 3 DIM)]. TRM2 = cows with at least one high heat index by 45 ± 3 DIM were monitored and received a $PGF_{2\alpha}$ treatment at 60 to 73 DIM, when they were 6 to 22 d after estrus. Cows were inseminated in estrus for 7 d and those not detected in estrus were enrolled in the hCG-Ovsynch, 67 to 80 DIM. TRM3 = cows that had had a high intensity estrus by 45 ± 3 DIM but where ≥23 d after estrus at 63 ± 3 DIM were enrolled in the hCG-Ovsynch. All cows were re-inseminated upon detection of estrus by an AMD.

According to the stochastic model, the mean (± SD) difference in adjusted gross profit was $8.69 ± $2.63/cow in favor of the TRM treatment, with 95% of the scenarios ranging from $4.39/cow to $13.03/cow (minimum = $−2.83/cow, maximum = $19.45/cow; Figure 4A). The variables with the lowest and highest Pearson correlation coefficients (Table 7) with the difference in adjusted gross profit between treatments were lactating cow feed cost (r = −0.394), value of Holstein female calf (r = −0.385), cost of hCG (r = −0.309), cow sale value (r = 0.234), replacement cost (r = 0.259), cost of GnRH and $PGF_{2\alpha}$ (r = 0.326), and value of crossbred calf (r = 0.542).

The mean (± SD) difference in adjusted gross profit between the TRM and control treatment among anestrus cows was $−10.67 ± 4.50/cow, according to the stochastic model. In 95% of the scenarios, the TRM treatment resulted in economic losses of $−18.08 to $−3.27/cow (minimum = $−29.05/cow, maximum = $8.36/cow; Figure 4B). Among anestrus cows, the variables with the lowest and highest Pearson correlation coefficients (Table 7) with the adjusted gross profit difference between treatments were milk price (r = −0.284), cost of hCG (r = −0.220), lactating cow feed cost (r = −0.209), cost of GnRH and $PGF_{2\alpha}$ (r = 0.163), value of crossbred calf (r = 0.288), and replacement cost (r = 0.828).

According to the stochastic model, the mean (± SD) difference in adjusted gross profit among estrual cows was $37.06 ± 5.61/cow in favor of the TRM treatment, with 95% of the scenarios ranging from $27.84/cow to $46.27/cow (minimum = $11.59/cow, maximum = $60.22/cow; Figure 4C). Among estrual cows, the variables with the lowest and highest Pearson correlation coefficient (Table 7) with adjusted gross profit difference between treatments were replacement cost (r = −0.608), value of Holstein female calf (r = −0.323), lactating cow feed cost (r = −0.212), cow sale value (r = 0.295), crossbred calf value (r = 0.308), and milk price (r = 0.480). Tornado graphs outlining the differences in economic outcomes between treatments are depicted in Figure 5A (overall), 5B (anestrus cows), and 5C (estrual cows).

DISCUSSION

In the current experiment, we aimed to evaluate whether a TRM, in which the reproductive hormone treatment was based on EPEC, would result in reproductive and economic performances not inferior to those obtained with the metaphylactic use of Double-Ovsynch for the first postpartum AI. In addition, we aimed to determine whether the use of a single hCG injection for the presynchronization of the estrus cycle of anestrus cows before the onset of the Ovsynch would produce P/AI that are not inferior to that of the Double-Ovsynch. A potential limitation of this experiment is the difference in the OvSP

Table 7. Pearson correlation coefficients between monetary values of variables used in the Monte Carlo stochastic analyses and adjusted gross profit difference between the TRM[1] and control[2] treatments according to 100,000 iterations

Variable	Overall r	Overall *P*-value	Anestrus[3] r	Anestrus[3] *P*-value	Estrual[4] r	Estrual[4] *P*-value
Inputs						
Milk price	0.131	<0.001	−0.284	<0.001	0.480	<0.001
Cow sale value	0.234	<0.001	−0.053	<0.001	0.295	<0.001
Female Holstein calf value	−0.385	<0.001	−0.097	<0.001	−0.323	<0.001
Male Holstein calf value	0.004	0.201	0.004	0.168	−0.001	0.8801
Crossbred calf value	0.542	<0.001	0.288	<0.001	0.308	<0.001
Value of primiparous' offspring	0.135	<0.001	0.068	<0.001	0.061	<0.001
Outputs						
Lactating cow feed cost	−0.394	<0.001	−0.209	<0.001	−0.212	<0.001
Dry cow feed cost	0.138	<0.001	0.021	<0.001	0.120	<0.001
Lactating cow fixed cost	−0.154	<0.001	−0.112	<0.001	−0.041	<0.001
Dry cow fixed cost	0.045	<0.001	−0.005	0.111	0.053	<0.001
Replacement cost	0.259	<0.001	0.828	<0.001	−0.608	<0.001
Reproductive management						
Holstein sex-sorted semen cost	0.008	0.013	−0.011	<0.001	0.023	<0.001
Beef semen cost	−0.014	<0.001	−0.002	0.561	−0.013	<0.001
Artificial insemination labor cost	−0.009	0.003	−0.006	0.055	−0.004	0.238
AMD cost	−0.003	0.278	−0.003	0.298	−0.0001	0.966
AMD fitting cost	−0.001	0.872	−0.001	0.648	0.001	0.777
GnRH and $PGF_{2\alpha}$ cost	0.326	<0.001	0.163	<0.001	0.186	<0.001
hCG cost	−0.309	<0.001	−0.220	<0.001	−0.100	<0.001
Labor cost to administer hormones	0.034	<0.001	0.017	<0.001	0.020	<0.001
Reproductive exam labor cost	−0.004	0.205	−0.004	0.212	0.001	0.698

[1]TRM: TRM1 = cows without an automated monitoring device (AMD) detected estrus with high heat index (primiparous ≥ 90, multiparous ≥ 70; 0 = minimum, 100 = maximum) by 45 ± 3 DIM were enrolled in the hCG-Ovsynch [2,000 IU of human chorionic gonadotropin at 56 ± 3 DIM and the Ovsynch protocol 7 d later (86 μg of gonadorelin at 63 ± 3 DIM, 500 μg of cloprostenol sodium at 70 ± 3 and 71 ± 3 DIM, 86 μg of gonadorelin at 72 ± 3 DIM, fixed time insemination at 73 ± 3 DIM)]. TRM2 = cows with at least one high heat index by 45 ± 3 DIM were monitored and received a $PGF_{2\alpha}$ treatment at 60 to 73 DIM, when they were 6 to 22 d after estrus. Cows were inseminated in estrus for 7 d and those not detected in estrus were enrolled in the hCG-Ovsynch, 67 to 80 DIM. TRM3 = cows that had had a high intensity estrus by 45 ± 3 DIM but where ≥23 d after estrus at 63 ±3 DIM were enrolled in the hCG-Ovsynch.

[2]Control: cows subjected to the Double-Ovsynch protocol (Souza et al., 2008) at 46 ± 3 DIM for first postpartum AI at fixed time (86 μg of gonadorelin at 46 ± 3 DIM, 500 μg of cloprostenol sodium at 53 ± 3, 86 μg of gonadorelin at 56 ± 3 and 63 ± 3 DIM, 500 μg of cloprostenol sodium at 70 ± 3 and 71 ± 3 DIM, 86 μg of gonadorelin at 72 ± 3 DIM, fixed time insemination at 73 ± 3 DIM).

[3]Cows without AMD-detected estrus with high heat index (primiparous ≥90, multiparous ≥70; 0 = minimum, 100 = maximum) by 45 ± 3 DIM.

[4]Cows with at least one AMD-detected estrus with high heat index (primiparous ≥90, multiparous ≥70; 0 = minimum, 100 = maximum) by 45 ± 3 DIM.

protocols between the control and TRM groups, where anestrus cows in the TRM group were presynchronized with hCG and those in the control group were submitted to Double-Ovsynch. Although this approach reflects the intended practical application, it introduces more nuances to the experiment than simply selecting cows based on EPEC to different reproductive managements. It is worth noting that this experiment was conducted in one herd in south-central Georgia using Holstein cows that calved in late summer and early fall and were, therefore, inseminated in late fall and early winter. Thus, the external validity of this experiment is limited.

Overall, cows metaphylactically subjected to the Double-Ovsynch protocol for first partum AI (control treatment) had P/AI 3.8 percentage points greater than those subjected to the TRM, independent of EPEC. When we analyzed the data from anestrus and estrual cows separately, we did not detect differences between the control and TRM treatments. This was likely due to the compartmentalization of the data resulting in a sample size that was insufficient to detect small differences. Still, the separate analyses of the data from anestrus and estrual cows are relevant because of the differences in reproductive management of cows in the TRM treatment according to EPEC. Among anestrus cows, the differences in P/AI between the control and TRM treatments at different time points ranged from 2.3 to 4.1 percentage points. Among estrual cows, the differences in P/AI between the control and TRM treatments at different time points ranged from 4.5 to 5.3 percentage points, and type of AI did not affect P/AI. The use of OvSP for TAI in dairy cattle is a common reproductive management strategy aimed at increasing the risk of AI. It synchronizes the recruitment of a new follicular wave, the luteolysis, and the timing of ovulation, thus allowing for the synchronized AI at a predetermined time (Pursley et al., 1995; Moreira et al., 2001; Vasconcelos et al., 1999). A few experiments have also reported that OvSP and TAI increase P/AI compared

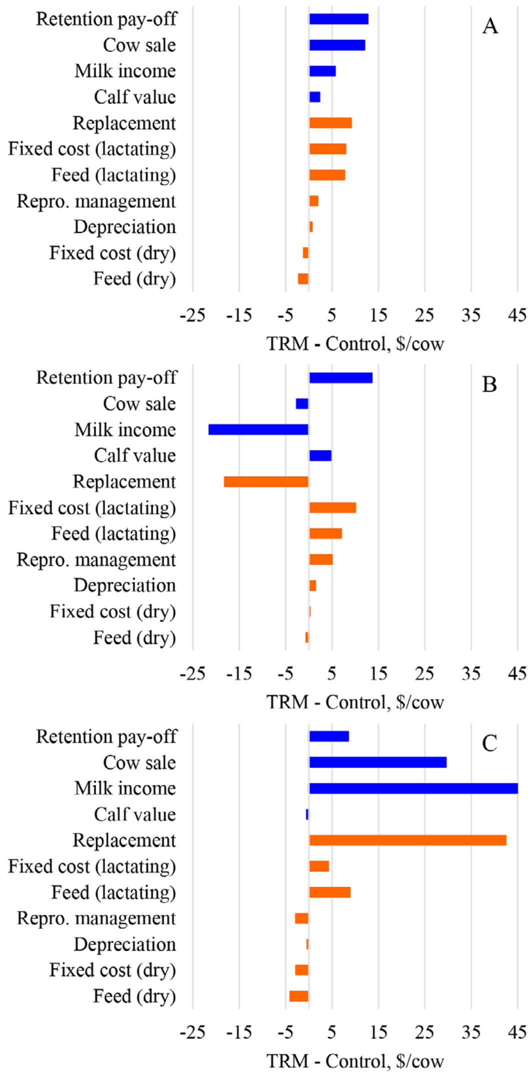

Figure 5. Tornado graphs showing the differences in monetary variables included in the stochastic simulation model. (A) Overall. (B) Anestrus cows: cows without automated monitoring device (AMD) detected high intensity estrus (primiparous ≥90, multiparous ≥70; 0 = minimum, 100 = maximum) by 45 ± 3 DIM. (C) Estrual cows: cows with at least one AMD-detected estrus with high heat index (primiparous ≥90, multiparous ≥70; 0 = minimum, 100 = maximum) by 45 ± 3 DIM. Control: cows subjected to the Double-Ovsynch protocol (Souza et al., 2008) at 46 ± 3 DIM for first postpartum AI at fixed time (86 µg of gonadorelin at 46 ± 3 DIM, 500 µg of cloprostenol sodium at 53 ± 3, 86 µg of gonadorelin at 56 ± 3 and 63 ± 3 DIM, 500 µg of cloprostenol sodium at 70 ± 3 and 71 ± 3 DIM, 86 µg of gonadorelin at 72 ± 3 DIM, fixed-time AI at 73 ± 3 DIM). TRM: TRM1 = cows without an AMD-detected estrus with high heat index (primiparous ≥90, multiparous ≥70; 0 = minimum, 100 = maximum) by 45 ± 3 DIM were enrolled in the hCG-Ovsynch (2,000 IU of human chorionic gonadotropin at 56 ± 3 DIM and the Ovsynch protocol 7 d later [86 µg of gonadorelin at 63 ± 3 DIM, 500 µg of cloprostenol sodium at 70 ± 3 and 71 ± 3 DIM, 86 µg of gonadorelin at 72 ± 3 DIM, fixed-time AI at 73 ± 3 DIM]). TRM2 = cows with at least one high heat index by 45 ± 3 DIM were monitored and received a $PGF_{2\alpha}$ treatment at 60 to 73 DIM, when they were 6 to 22 d after estrus. Cows were inseminated in estrus for 7 d and those not detected in estrus were enrolled in the hCG-Ovsynch, 67 to 80 DIM. TRM3 = cows that had had a high intensity estrus by 45 ± 3 DIM but where ≥23 d after estrus at 63 ± 3 DIM were enrolled in the hCG-Ovsynch. All cows were re-inseminated upon detection of estrus by an AMD. Repro. = reproductive.

with AI at estrus, likely by improving the hormonal milieu and timing of AI relative to ovulation (Santos et a., 2017; Sitko et al., 2023). Our group also demonstrated that primiparous cows that had had an AMD-detected estrus by 50 DIM subjected to the Double-Ovsynch and TAI had greater P/AI than those AI at AMD-detected estrus, but not multiparous cows (Gonzalez et al., 2023). Ovulation synchronization protocols and TAI have been widely adopted by the dairy industry across the United States with varying degrees of success and, for the most part, the dairy industry has benefited from it.

Giordano et al. (2012b) had demonstrated that the use of one hCG injection to presynchronize the Ovsynch protocol produced similar P/AI in re-inseminated cows compared with the Double-Ovsynch, along with reducing the inter-AI interval by 10 d. In the current experiment, the P/AI was slightly lower among anestrus cows that had their estrus cycle presynchronized with the hCG protocol compared with presynchronization with the Ovsynch protocol. Human chorionic gonadotropin mimics the action of LH by binding to LH receptors on the dominant follicle, inducing its final maturation and ovulation while bypassing the pituitary, unlike GnRH that relies on the cow's endogenous pituitary response to release LH. This leads to a more immediate and consistent ovulatory response, which can be particularly advantageous in cows with compromised pituitary function or when the LH surge is insufficient (Gumen et al., 2022; Ireland and Roche, 1982; Ireland and Roche, 1983). Although cows that had had their estrus cycle presynchronized with hCG were less likely to have a CL on the day of the first GnRH of the Ovsynch protocol, the hCG presynchronization resulted in greater progesterone concentration on the day of the first GnRH of the Ovsynch protocol. Once ovulation is induced by GnRH and hCG, a new CL is formed but the prolonged luteotropic effect of hCG (Vasconcelos et al., 2018) may have caused increased progesterone synthesis from the newly formed and existing CL. Rispoli and Nett (2005) demonstrated that progesterone may decrease the estradiol-induced expression of GnRH receptors in the pituitary tissue of sheep, potentially attenuating the pituitary's responsiveness to GnRH. Thus, the greater progesterone concentration at the start of the Ovsynch in cows that had their estrus presynchronized with hCG could explain why they were less likely to have a CL and had lower progesterone concentration at the time of $PGF_{2\alpha}$ treatment. Still, the differences in ovarian dynamics and progesterone concentration between the TRM and control treatments among anestrus cows resulted in only minute differences in P/AI.

The concept of TRM comes from the realization that cows that have AMD-detected estrus during the early postpartum period have greater P/AI to first service and fewer days open than cows with no estrus events (Borchardt et al., 2021; Rial et al., 2022). This corroborates the findings from other earlier research demonstrating that acyclicity of the ovaries during early postpartum negatively affects the P/AI to first AI and the long-term reproductive performance of cows (Santos et al., 2009). In our experiment we classified cows as anestrus and estrual based on EPEC, not based on methods that are considered gold-standard (e.g., serum progesterone concentrations) or have been validated (e.g., ovarian ultrasound examinations) for diagnosis of resumption of cyclicity. Borchardt et al. (2023) demonstrated that AMD have 84.0% and 34.1% sensitivity and specificity, respectively, for the detection of resumption of ovarian cyclicity compared with circulating progesterone concentration. Thus, we expect that our classification of estrual cows more closely reflected the resumption of ovarian cyclicity than our classification of anestrus cows reflected the absence of ovarian cyclicity. This is an important caveat because misclassification of anestrus cows would likely be less consequential than misclassification of estrual cows because all of the former were enrolled in OvSP for TAI.

Fricke et al. (2014) and Rial et al. (2022) compared the P/AI of cows metaphylactically subjected to the Presynch-Ovsynch or Double-Ovsynch, respectively, to that of cows that were AI at AMD-detected estrus according to their EPEC and enrolled in the OvSP if not AI in estrus. In both instances the P/AI of cows metaphylactically subjected to the OvSP was greater than that of cows managed according to their EPEC, but the hazard of pregnancy was not different between treatments (Fricke et al., 2014) or was greater for cows managed according to their EPEC (Rial et al., 2022). As mentioned previously, our group also demonstrated that while P/AI of primiparous cows managed according to EPEC was lower than that of cows metaphylactically subjected to the Double-Ovsynch, this was not true for multiparous cows (Gonzalez et al., 2023). In the current experiment, we determined that the hazard of pregnancy over the entire lactation was lower for estrual cows in the TRM treatment, but not among anestrus cows.

Efficient reproductive management is crucial for the economic success of dairy operations, as it influences milk production efficiency (e.g., IOFC), longevity, the availability of replacement animals, and culling policies. Because in our experiment the difference in the hazard of pregnancy was not large, culling dynamics were not affected, as seen by the lack of differences between treatments in the likelihood of cows starting a new lactation and being sold. Similarly, DIM, milk yield, and estimated DMI were not affected, resulting in no differences between treatments in gross profit and adjusted gross profit. The lack of interactions between treatment and parity and between treatment and EPEC further underscore that the TRM for first postpartum AI adopted in the current experiment can be a viable alternative to the metaphylactic use of OvSP and TAI. The stochastic models resulted in minute economic advantages toward the TRM treatment that were mainly explained by advantages toward the TRM over the control treatment among estrual cows (anestrus = −$10.7, estrual = $37.1). Among anestrus cows, RPO was $13.6/cow greater and milk income was $21.5/cow lower for the TRM treatment, whereas replacement cost was $18.2/cow lower, and fixed costs, lactating cow feed costs, and reproductive management costs were $10/cow, $7/cow, and $5/cow, respectively, greater for the TRM treatment. Conversely, among estrual cows, RPO, cow sale income, and milk income were $8.5/cow, $29.5, and $45/cow, respectively, greater for the TRM treatment, but replacement cost was $42.4/cow greater for the TRM treatment. In a recent experiment, we demonstrated that management of first postpartum AI according to EPEC and re-AI throughout the lactation in estrus with the aid of AMD increased gross profit by $108/cow, primarily due to improvements in the hazard of re-AI and changes in culling dynamics (Gonzalez et al., 2023). Sitko et al. (2023) evaluated the cash flow of primiparous cows managed with AMD-aided estrus detection versus a hormone-based protocol and found no clear economic advantage because the differences in reproductive performance were small and did not affect calving interval and culling dynamics. In the current experiment, the differences in economic outcomes between the actual data from the collaborating herd and the stochastic model likely resulted from differences in the monetary values for the variables used in the 2 scenarios. Stochastic models are important in economics because they account for uncertainty and randomness in economic systems, enabling more accurate predictions, better decision-making, and the realistic analysis of dynamic, unpredictable events. Additional experiments exploring TRM should continue to explore its impacts on the economic outcomes to inform dairy producers on the most profitable strategies.

The finding that anestrus cows had ~16% and 11% lower P/AI after the first and second postpartum AI, respectively, is consistent with previous studies showing the importance of resumption of ovarian cyclicity postpartum (Santos et al., 2009). As a consequence of the reduced P/AI early in lactation, anestrus cows had reduced hazard of pregnancy. This impaired reproductive performance led to a change in culling practices with more anestrus cows deemed not eligible for AI and sold and fewer of them starting a new lactation than estrual cows. Conversely, anestrus cows had greater total milk yield and feed intake during their lactation and had greater estimated BW at dry-off, longer dry period, and greater intake during the dry period. Despite these differences in zootechnical responses, anestrus cows only had a slightly greater IOFC than estrual cows, whereas the gross profit and adjusted gross profit did not differ.

CONCLUSIONS

Our experiment evaluated the viability of a TRM based on EPEC compared with the metaphylactic use of the Double-Ovsynch protocol for first postpartum AI. We also assessed whether a single presynchronizing injection of hCG before the Ovsynch would result in P/AI not inferior to the Double-OVsynch among anestrus cows. While the Double-Ovsynch yielded slightly higher P/AI, economic analysis showed minimal differences between treatments. Differences in P/AI early in lactation did not translate into long-term differences in reproductive performance or culling rates. This suggests that reproductive strategies for first AI can be tailored to individual cows without significant economic losses when the hazard of re-AI and pregnancy to re-AI are high. The TRM protocol provides flexibility, particularly for estrual cows, allowing for reduced hormone use while maintaining reproductive performance. Future research should explore TRM in diverse herds and environments to confirm its broader applicability. By refining reproductive strategies, it may be possible to offer producers cost-effective methods to improve herd fertility while promoting judicious use of hormones.

NOTES

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Nonstandard abbreviations used: AI = artificial insemination; AMD = automated monitoring device; AOR = adjusted OR; dist. = distribution; EPEC = early postpartum estrus characteristics; gDPR = daughter pregnancy rate; hCG = human chorionic gonadotropin; HI = heat index; IOFC = income over feed cost; max. = maximum; min. = minimum; OvSP = ovulation synchronization protocol; preg. = pregnant; reprod. = reproductive; RPO = retention payoff; TAI = timed AI; THI = temperature-humidity index; TRM = targeted reproductive management; TRM1 = cows that did not have an AMD-detected estrus with high heat index (primiparous ≥90, multiparous ≥70; 0 = minimum, 100 = maximum) by 45 ± 3 DIM were enrolled in the hCG-Ovsynch (hCG at 56 ± 3 DIM, GnRH at 63 ± 3 DIM, $PGF_{2\alpha}$ at 70 ± 3 and 71 ± 3 DIM, GnRH at 72 ± 3 DIM, TAI at 73 ± 3 DIM); TRM2 = cows with at least one high heat index AMD-detected estrus by 45 ± 3 DIM received a $PGF_{2\alpha}$ treatment at 60 to 73 DIM when they were 6 to 22 d after a previously recorded estrus. Cows were monitored for signs of estrus for 7 d after the $PGF_{2\alpha}$ treatment; cows detected in estrus were AI on the same day, whereas those not detected in estrus were enrolled in the hCG-Ovsynch 67 to 80 DIM; TRM3 = cows that had had at least one high heat index AMD-detected estrus by 45 ± 3 DIM but where ≥23 d from a previously recorded estrus at 63 ± 3 DIM were enrolled in the hCG-Ovsynch at 63 ± 3 DIM.

REFERENCES

Bello, N. M., J. P. Steibel, and J. R. Pursley. 2006. Optimizing ovulation to first GnRH improved outcomes to each hormonal injection of Ovsynch in lactating dairy cows. J. Dairy Sci. 89:3413–3424. https://doi.org/10.3168/jds.S0022-0302(06)72378-5.

Borchardt, S., T. A. Burnett, W. Heuwieser, J. L. Plenio, R. S. Conceição, R. L. A. Cerri, and A. M. L. Madureira. 2023. Efficacy of an automated technology at detecting early postpartum estrus events: Can we detect resumption of cyclicity? JDS Commun. 5:225–229. https://doi.org/10.3168/jdsc.2023-0463.

Borchardt, S., C. M. Tippenhauer, J.-L. Plenio, A. Bartel, A. M. L. Madureira, R. L. A. Cerri, and W. Heuwieser. 2021. Association of estrous expression detected by an automated activity monitoring system within 40 days in milk and reproductive performance of lactating Holstein cows. J. Dairy Sci. 104:9195–9204. https://doi.org/10.3168/jds.2020-19705.

Cabrera, V. E. 2012. A simple formulation and solution to the replacement problem: A practical tool to assess the economic cow value, the value of a new pregnancy, and the cost of a pregnancy loss. J. Dairy Sci. 95:4683–4698. https://doi.org/10.3168/jds.2011-5214.

Cerri, R. L., H. M. Rutigliano, R. C. Chebel, and J. E. Santos. 2009. Period of dominance of the ovulatory follicle influences embryo quality in lactating dairy cows. Reproduction 137:813–823. https://doi.org/10.1530/REP-08-0242.

Chebel, R. C., J. E. Santos, R. L. Cerri, H. M. Rutigliano, and R. G. Bruno. 2006. Reproduction in dairy cows following progesterone insert presynchronization and resynchronization protocols. J. Dairy Sci. 89:4205–4219. https://doi.org/10.3168/jds.S0022-0302(06)72466-3.

Denicol, A. C., G. Lopes Jr., L. G. Mendonça, F. A. Rivera, F. Guagnini, R. V. Perez, J. R. Lima, R. G. Bruno, J. E. Santos, and R. C. Chebel. 2012. Low progesterone concentration during the development of the first follicular wave reduces pregnancy per insemination of lactating dairy cows. J. Dairy Sci. 95:1794–1806. https://doi.org/10.3168/jds.2011-4650.

De Vries, A. 2006. Economic value of pregnancy in dairy cattle. J. Dairy Sci. 89:3876–3885. https://doi.org/10.3168/jds.S0022-0302(06)72430-4.

Ferguson, J. D., D. T. Galligan, and N. Thomsen. 1994. Principal descriptors of body condition score in Holstein cows. J. Dairy Sci. 77:2695–2703. https://doi.org/10.3168/jds.S0022-0302(94)77212-X.

Fricke, P. M., J. O. Giordano, A. Valenza, G. Lopes Jr., M. C. Amundson, and P. D. 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. https://doi.org/10.3168/jds.2013-7366.

Galvão, K. N., P. Federico, A. De Vries, and G. M. Schuenemann. 2013. Economic comparison of reproductive programs for dairy herds using estrus detection, timed artificial insemination, or a combination. J. Dairy Sci. 96:2681–2693. https://doi.org/10.3168/jds.2012-5982.

Galvão, K. N., M. F. Sá Filho, and J. E. Santos. 2007. Reducing the interval from presynchronization to initiation of timed artificial insemination improves fertility in dairy cows. J. Dairy Sci. 90:4212–4218. https://doi.org/10.3168/jds.2007-0182.

Giordano, J. O., A. S. Kalantari, P. M. Fricke, M. C. Wiltbank, and V. E. Cabrera. 2012a. A daily herd Markov-chain model to study the reproductive and economic impact of reproductive programs combining timed artificial insemination and estrus detection. J. Dairy Sci. 95:5442–5460. https://doi.org/10.3168/jds.2011-4972.

Giordano, J. O., M. C. Wiltbank, J. N. Guenther, M. S. Ares, G. Lopes Jr., M. M. Herlihy, and P. M. Fricke. 2012b. Effect of presynchronization with human chorionic gonadotropin or gonadotropin-releasing hormone 7 days before resynchronization of ovulation on fertility in lactating dairy cows. J. Dairy Sci. 95:5612–5625. https://doi.org/10.3168/jds.2011-5035.

Gonzalez, T. D., L. Factor, A. Mirzaei, A. B. Montevecchio, S. Casaro, V. R. Merenda, J. G. Prim, K. N. Galvão, R. S. Bisinotto, and R. C. Chebel. 2023. Targeted reproductive management for lactating Holstein cows: Reducing the reliance on exogenous reproductive hormones. J. Dairy Sci. 106:5788–5804. https://doi.org/10.3168/jds.2022-22666.

Gumen, A., A. Kaya, and M. Yilmazbas. 2022. Comparative analysis of hCG and GnRH for ovulation synchronization in dairy cattle. Theriogenology 178:14–22. https://doi.org/10.1016/j.theriogenology.2024.05.030.

Ireland, J. J., and J. F. Roche. 1982. Development of antral follicles in cattle after prostaglandin-induced luteolysis: Changes in serum hormones, steroids in follicular fluid, and gonadotropin receptors. Endocrinology 111:2077–2086.

Ireland, J. J., and J. F. Roche. 1983. Growth and differentiation of large antral follicles after spontaneous luteolysis in heifers: Changes in concentration of hormones in follicular fluid and specific binding of gonadotropins to follicles. J. Anim. Sci. 57:157–167.

Mader, T. L., M. S. Davis, and T. M. Brown–Brandl. 2006. Environmental factors influencing heat stress in feedlot cattle. J. Anim. Sci. 84:712–719. https://doi.org/10.2527/2006.843712x.

Megahed, A. A., K. L. Jones, R. S. Bisinotto, R. C. Chebel, K. N. Galvão, A. M. Chan, and J. H. J. Bittar. 2023. Validation of a fully automated chemiluminescent immunoassay for cattle serum and plasma progesterone measurement. Front. Vet. Sci. 9:1064201. https://doi.org/10.3389/fvets.2022.1064201.

Moreira, F., C. Orlandi, C. A. Risco, R. Mattos, F. Lopes, and W. W. Thatcher. 2001. Effects of presynchronization and bovine somatotropin on pregnancy rates to a timed artificial insemination protocol in lactating dairy cows. J. Dairy Sci. 84:1646–1659. https://doi.org/10.3168/jds.S0022-0302(01)74600-0.

NRC. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. Natl. Acad. Sci., Washington, DC.

NRC. 2021. Nutrient Requirements of Dairy Cattle. 8th rev. ed. Natl. Acad. Sci., Washington, DC.

Olynk, N. J., and C. A. Wolf. 2007. Expected net present value of pure and mixed sexed semen artificial insemination strategies in dairy heifers. J. Dairy Sci. 90:2569–2576. https://doi.org/10.3168/jds.2006-460.

Pursley, J. R., M. O. Mee, and M. C. Wiltbank. 1995. Synchronization of ovulation in dairy cows using $PGF_{2\alpha}$ and GnRH. Theriogenology 44:915–923. https://doi.org/10.1016/0093-691x(95)00279-h.

Rial, C., A. Laplacette, and J. O. Giordano. 2022. Effect of a targeted reproductive management program designed to prioritize insemination at detected estrus and optimize time to insemination on the reproductive performance of lactating dairy cows. J. Dairy Sci. 105:8411–8425. https://doi.org/10.3168/jds.2022-22082.

Rispoli, L. A., and T. M. Nett. 2005. Pituitary gonadotropin-releasing hormone (GnRH) receptor: Interaction with estradiol and GnRH in sheep. Biol. Reprod. 72:503–508.

Santos, J. E., H. M. Rutigliano, and M. F. Sá Filho. 2009. Risk factors for resumption of postpartum estrous cycles and embryonic survival in lactating dairy cows. Anim. Reprod. Sci. 110:207–221. https://doi.org/10.1016/j.anireprosci.2008.01.014.

Santos, V. G., P. D. Carvalho, C. Maia, B. Carneiro, A. Valenza, and P. M. Fricke. 2017. Fertility of lactating Holstein cows submitted to a Double-Ovsynch protocol and timed artificial insemination versus artificial insemination after synchronization of estrus at a similar day in milk range. J. Dairy Sci. 100:8507–8517. https://doi.org/10.3168/jds.2017-13210.

Schulz, L. 2020. Historical cattle prices. Ag Decision Maker. Extension and Outreach, Iowa State University. Accessed Nov. 19, 2024. https://www.extension.iastate.edu/agdm/livestock/pdf/b2-12.pdf.

Sitko, E. M., F. A. Di Croce, A. K. McNeel, D. J. Weigel, and J. O. Giordano. 2023. Effect of reproductive management programs that prioritized artificial insemination at detected estrus or timed artificial insemination on the economic performance of primiparous Holstein cows of different genetic merit for fertility. J. Dairy Sci. 106:6495–6514. https://doi.org/10.3168/jds.2022-22674.

Souza, A. H., H. Ayres, R. M. Ferreira, and M. C. Wiltbank. 2008. A new presynchronization system (Double-Ovsynch) increases fertility at first postpartum timed AI in lactating dairy cows. Theriogenology 70:208–215.

USDA-ERS. 2023. Feed price. National feed cost production. Accessed Jun. 20, 2024. https://www.ers.usda.gov/data-products/milk-cost-of-production-estimates/milk-cost-of-production-estimates/#Recent%20Milk%20Cost%20of%20Production%20Estimates-2016%20Base.

USDA-NASS. 2022a. Milk Price. Accessed Sep. 19, 2022. https://quickstats.nass.usda.gov/results/70826CF5-A4E4-3264-823D-FD0E20C531F7.

USDA-NASS. 2022b. Beef Price. Accessed Jun. 20, 2024. https://quickstats.nass.usda.gov/results/4EAF312F-59BA-3504-820C-415D1D7CECFE.

USDA-NASS. 2022c. Replacement Price. Accessed Jun. 20, 2024. https://quickstats.nass.usda.gov/results/98409749-B223-3ECC-ADDD-42984391CB2E.

USDA-NASS. 2022d. Labor Price. Accessed Jun. 20, 2024. https://quickstats.nass.usda.gov/results/C036B1BE-653C-392A-865A-714795334E81.

USDA-NASS. 2022e. Southern Region News Release Farm Labor. Accessed Jun. 20, 2024. https://www.nass.usda.gov/Statistics_by_State/Regional_Office/Southern/includes/Publications/Economic_and_Demographic_Releases/Farm_Labor/2022/AprilLabor2022.pdf.

van Arendonk, J. A. M. 1985. A model to estimate the performance, revenues and costs of dairy cows under different production and price situations. Agric. Syst. 16:157–189. https://doi.org/10.1016/0308-521X(85)90010-1.

Vasconcelos, J. L., R. W. Silcox, G. J. Rosa, J. R. Pursley, and M. C. Wiltbank. 1999. Synchronization rate, size of the ovulatory follicle, and pregnancy rate after synchronization of ovulation beginning on different days of the estrous cycle in lactating dairy cows. Theriogenology 52:1067–1078. https://doi.org/10.1016/S0093-691X(99)00195-8.

Vasconcelos, J. L. M., A. B. Nascimento, R. D. Oliveira, F. S. Mesquita, M. O. Marques, F. Morotti, R. M. Santos, L. D. P. Sinedino, and J. E. P. Santos. 2018. Strategies to improve pregnancy per artificial insemination in lactating dairy cows based on circulating progesterone concentration before AI. J. Dairy Sci. 101:4598–4608. https://doi.org/10.3168/jds.2017-13476.