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Original Research Article

Effect of a targeted reproductive management program based on automated detection of estrus during the voluntary waiting period on reproductive performance of lactating dairy cows

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ABSTRACT

The objective of this experiment was to evaluate the effect on reproductive performance of a targeted reproductive management (**TRM**) program for first postpartum insemination (**AI**) that prioritized AI at detected estrus (**AIE**) by providing different intervals for estrus detection based on records of automated estrus alerts (**AEA**) during the voluntary waiting period (**VWP**). A secondary objective was to evaluate the association between occurrence of AEA during the VWP and reproductive performance. Lactating Holstein cows (n = 1,260) fitted with neck behavior monitoring sensors for detection of estrus were randomly assigned to a program that used all-timed AI (**TAI**) for first service (**ALL-TAI**; n = 632) or a TRM program that prioritized AIE and used TAI only for cows not detected in estrus (**TP-AIE**; n = 628). Cows in the ALL-TAI treatment received TAI at 76 ± 3 days in milk (**DIM**) after a Double-Ovsynch protocol. Cows in the TP-AIE treatment were eligible for AIE for 30 ± 3 or 16 ± 3 d after a 49 d VWP if at least one (n = 346) or no (n = 233) AEA were recorded from 15 to 49 DIM. Cows not AIE received TAI after an Ovsynch protocol with progesterone supplementation at 90 ± 3 or 76 ± 3 DIM if the cow had or did not have AEA during the VWP, respectively. Data were analyzed by logistic and Cox's proportional hazard regression. In the TP-AIE treatment, 69.3 % of cows received AIE and more cows with (83.3 %) than without (45.0 %) AEA during the VWP received AIE. Cows in the TP-AIE (69.0 ± 0.7 d) treatment had fewer days from calving to first AI than cows in the ALL-TAI (75.7 ± 0.8 d) treatment. The proportion of cows pregnant by 150 DIM (ALL-TAI = 59.1 % and TP-AIE = 56.0 %) and the hazard ratio (**HR**) for time to pregnancy (1.0 [95 % confidence interval: 0.9, 1.2]) did not differ between treatments and median days to pregnancy were 102 and 107 for the ALL-TAI and TP-AIE treatments, respectively. Overall, the ALL-TAI (42.3 %) treatment had more first service pregnancies per AI (**P/AI**) than the TP-AIE (29.0 %) treatment. Cows with AEA during the VWP had greater P/AI (42.5 % vs. 28.9 %), proportion of cows pregnant by 150 DIM (67.4 % vs. 47.0 %), and HR for time to pregnancy (1.6 [1.4, 1.9]) than cows without AEA during the VWP. We conclude that a TRM program that prioritized AIE based on AEA during the VWP led to a similar pregnancy rate and proportion of cows pregnant by mid-lactation than a program that used all-TAI with extended VWP despite fewer P/AI to first service. Also, expression of estrus during the VWP was associated with improved reproductive performance. Thus, AEA during the VWP could be used as a predictor of reproductive potential for TRM of lactating dairy cows.

1. Introduction

Targeted reproductive management (**TRM**) programs have been recently explored as an alternative approach to whole herd reproductive management [1–3]. This approach consists of identifying cows with different reproductive performance potential using one or more sources of biological, management and performance data as predictors. Subgroups of individuals that share similar biological features or expected performance can then receive management strategies specifically designed to optimize performance, management, profitability, or

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combinations thereof [4]. Ultimately, optimized outcomes for subgroups of cows targeted with different management strategies are expected to enhance overall herd performance or management to levels not achievable when the whole herd is managed with a single reproductive program.

Increased adoption by dairy farms of automated monitoring systems that collect cow- and herd-level physiological, performance, and behavioral parameters for predicting discrete events, such as estrus, is enabling the development of novel TRM programs [3–5]. Recent studies reported that automated estrus alerts (**AEA**) from automated monitoring systems can help identify cows with a different probability of expressing estrus and fertility after the end of the voluntary waiting period (**VWP**) [3,6–8]. Identifying subgroups of cows with different likelihood of expressing estrus and fertility potential can then be used to assign cows to reproductive programs that optimize aspects of performance or herd management based on the ability of cows to express estrus and become pregnant if inseminated at detected estrus (**AIE**). For example, cows most likely to succeed if submitted to AIE can be assigned to programs that promote expression of estrus and reduce hormonal interventions for timed AI (**TAI**). Conversely, cows expected to be less successful, if managed with programs that prioritize AIE, could benefit from more aggressive use of TAI with synchronization of ovulation protocols known to increase fertility of cows not AIE after a period of detection of estrus [4,5,9].

Despite evidence that expression of estrus during the VWP of lactating dairy cows is associated with future reproductive performance, a few experiments evaluated the effect of using estrus alerts during the VWP to create subgroups of cows for TRM [1,3,8]. These randomized control experiments compared the performance of first service TRM programs in which cows were either eligible for AIE for longer periods of time if they had at least one AEA during the VWP [1,3] or cows without AEA during the VWP were eligible for AIE for shorter periods of time. Cows not detected in estrus in either group received TAI after synchronization of ovulation. The TRM programs in these experiments resulted in reduced first service P/AI but a similar pregnancy rate than programs known to maximize first service fertility through all-TAI and an extended VWP. Moreover, at least in one experiment [3], a TRM program based on AEA during the VWP was as effective as a non-TRM program that prioritized AIE but did not optimize use of TAI based on AEA during the VWP. Although, these experiments demonstrated that targeted management based on AEA during the VWP can be as efficient for generating pregnancies during lactation as other effective reproductive management programs, more research is needed to evaluate the effect of this approach to herd management under a wide range of estrus detection efficiency and P/AI, using different automated estrus detection systems, and under a wide range of herd management and environmental conditions. A better understanding of the associations between expression of estrus during the VWP with other reproductive physiological outcomes and reproductive performance is also needed. This is especially relevant as the increased adoption of automated estrus detection (**AED**) systems facilitates cow grouping for implementation of TRM strategies through real-time integration of data from predictors of reproductive performance such as AEA [4].

Thus, we hypothesized that a TRM program designed to optimize the combination of AIE and TAI by providing a different duration of the period of detection of estrus to cows with than without AEA during the VWP would result in a similar pregnancy dynamic than a program that used all-TAI after a Double-Ovsynch protocol and an extended VWP. Secondary hypotheses were that physiological features and cyclicity status during the VWP would be associated with expression of estrus and detection through AEA, and that cows with AEA during the VWP would have better reproductive performance than cows with no AEA. Therefore, the primary objective of this experiment was to evaluate the effect of a TRM program that prioritized AIE based on AEA during the VWP on the reproductive performance of lactating dairy cows by 150 DIM. Our secondary objectives were to evaluate the association between cow physiological features and cyclicity status with the likelihood of detection of estrus based on AEA during the VWP and reproductive outcomes for cows with or without AEA during the VWP.

2. Materials and methods

All experimental procedures conducted with animals were approved by the Animal Care and Use Committee of Cornell University (Protocol # 2021- 0031).

2.1. Farm and cow management

This experiment was conducted on a commercial dairy farm located in Tequisquiapan, Mexico from February of 2021 to June of 2022. The farm housed an average of 1257 (range 1210 to 1311) milking cows and 188 (range 115–231) dry cows during the study period.

During the prepartum period, cows were housed in nulliparous and parous cow groups in bedded pack pens with grooved concrete alleys and recycled manure solids as bedding. After parturition, cows were milked for the first time in a separate parlor in the prepartum area and then transported to lactating cow barns. Cows remained in fresh pens separated by parity (primiparous and multiparous) until ~15 DIM. All cows received 500 μg of Cloprostenol sodium (Celosil, MSD Salud Animal, Ciudad de Mexico, Mexico) at 17 ± 3 DIM. After 15 DIM, cows were moved to primiparous and multiparous high-production group pens until ~180 DIM when cows were transferred to medium-production groups until dry off. Housing for lactating cows consisted of 4-row freestall barns with deep-sand bedded stalls with access to open dry-lots with dirt and recycled manure solids. Dry-lots were open all day when weather conditions were favorable and closed on rainy days. No cooling system for heat abatement was available.

Milking occurred 3 × /d at ~8 h intervals (0600–1000 h, 1400–1800 h, and 2200 to 0200 h) in an external 60-point rotary parlor (Afimilk MPC, Afimilk Ltd., Kibbutz Afikim, Israel). Cows had ad libitum access to water and a TMR delivered 1 × /d for far-off, close-up, and fresh pens and 3 × /d for high and medium production pens. Diets provided during the prepartum and lactating periods were the same for primiparous and multiparous cows.

2.2. Automated behavior monitoring system for detection of estrus

Cows were fitted with a neck behavior monitoring sensor tag (SenseHub Monitoring Neck Tag, Merck Animal Health, Rahway, NJ, USA) a minimum of one month before expected parturition to ensure data was collected for at least 7 d for physical activity and 10 d for rumination time, per the manufacturer requirements.

Physical activity (min/h) and rumination time (min/h) were collected continuously by the sensor tag and transferred every 20 min through antennas (SenseHub Antenna, Merk Animal Health, Rahway, NJ, USA) to the system server for processing and storage (DataFlow II Monitoring, Merck Animal Health, Rahway, NJ, USA). Using an internal algorithm, the software combined data for physical activity and rumination time to generate estrus alerts. All AEA had a Heat Index value that indicated the probability of estrus. A Heat Index above 32 triggered an estrus alert. Cows were included in a report for insemination once the activity increase reached its zenith (i.e., maximum value). Once a zenith was reached, a 26 h countdown indicative of the expected time of ovulation began. Cows were eligible for insemination when the countdown was between 23 and 7 h. Two times per day (0800 h and 1400 h), farm personnel printed the report with cows for insemination. Due to the occurrence of false positive AEA when cows are moved between pens at commercial farms [3], all AEA recorded on the day of or the day after a pen move were considered false positives and ignored. All cows with an AEA were examined by farm personnel to confirm estrus after the first (0800–1000 h) or the second milking session of the day (1400–1800 h) if the cow was present on the estrus report list at 0800 or 1400 h, respectively. In this examination, uterine tone was evaluated through rectal palpation and the presence of vaginal mucus discharge, redness of the vulva, and signs of mounting or rubbing were evaluated by visual observation.

2.3. Experimental design

All cows that initiated lactation and were eligible for pregnancy were enrolled in the experiment which followed a randomized complete block design. Parity (primiparous vs. multiparous) and type of semen assigned for first service (Holstein vs. beef) were used as blocking factors. Following farm operating procedures, cows in the first and second lactation were assigned to AI with conventional Holstein semen whereas cows in third and greater lactation were assigned to AI with conventional beef semen (Angus and Gyr).

Weekly, research personnel (A. L. L.) generated the list of cows at 6 ± 3 DIM that were eligible for enrollment and then randomly assigned eligible cows to one of two experimental treatments (n = 1260). All AEA from 15 DIM to the end of the VWP at 49 DIM were recorded for assigning cows into estrus groups (**E-group**) based on the presence of at least one AEA (**E-VWP**) or no AEA (**NE-VWP**) (Fig. 1). Cows were assigned to a control treatment that consisted of first service TAI with an extended VWP of 76 ± 3 DIM for all cows [**ALL-TAI**; n = 632 (primiparous: n = 243 and multiparous: n = 389 and Holstein semen: n = 423 and beef semen: n = 209)] after synchronization of ovulation with the Double-Ovsynch protocol with two $PGF_{2\alpha}$ treatments (GnRH, 7 d later $PGF_{2\alpha}$, 3 d later GnRH, 7 d later GnRH, 7 d later $PGF_{2\alpha}$, 24 h later $PGF_{2\alpha}$, 32 h later GnRH, and 16 h later TAI). Cows assigned to the experimental treatment were submitted to first service with a TRM program [**TP-AIE**, n = 628 (primiparous: n = 243 and multiparous: n = 385; Holstein semen: n = 420 and beef semen: n = 208)] with VWP of 50 DIM. In the TRM treatment, cows in the E-VWP and NE-VWP group were provided with different lengths of time after the end of the VWP for submission to AIE. Cows in the E-VWP were eligible to receive AIE for 30 ± 3 d whereas cows in the NE-VWP were eligible to receive AIE for 16 ± 3 d after the end of VWP. Thereafter, cows not submitted to AIE were enrolled in an Ovsynch protocol with progesterone (**P4**) supplementation and an additional $PGF_{2\alpha}$ treatment (**P4-Ov**; GnRH and intravaginal P4-releasing device, 7 d later $PGF_{2\alpha}$ and P4-releasing device removal, 24 h later $PGF_{2\alpha}$, 32 h later GnRH, and 16 h later TAI) and submitted to TAI at 90 ± 3 DIM or 76 ± 3 DIM for the E-VWP and NE-VWP group, respectively.

All GnRH treatments consisted of an i.m. injection of 100 μg of Gonadorelin diacetate (Fertagyl, MSD Salud Animal, Ciudad de Mexico, Mexico), all $PGF_{2\alpha}$ treatments consisted of an i.m. injection of 500 μg of Cloprostenol sodium (Celosil, MSD Salud Animal, Ciudad de Mexico, Mexico), and P4 supplementation was provided by a intravaginal P4 releasing device containing 1.3 g of P4 (CRESTAR®IVG Intravaginal 1.3, MSD Salud Animal, Ciudad de Mexico, Mexico).

All AIE services for cows in the TP-AIE treatment were performed if cows had an AEA, as previously defined. Inseminations after AEA were conducted 2 × /d (0900–1100 h and 1500–1630 h) by one of three farm technicians. Timed AI was conducted 1 × /d on Tuesdays (0900–1100 h) by one of three farm technicians in cows that completed the synchronization of ovulation protocols.

Pregnancy diagnosis was conducted by the farm veterinarian via transrectal ultrasonography of the reproductive tract at 34 ± 3 d after AI and confirmation of pregnancy was performed at 62 ± 3 d after AI by rectal palpation of the uterus. Pregnancy loss was calculated from the day of the initial pregnancy diagnosis until confirmation of pregnancy. A pregnancy loss was recorded when a cow diagnosed pregnant at the first examination was either not pregnant at the time of confirmation or was reinseminated between the two pregnancy exams.

Cows from both treatments received second and subsequent AIE services with the same management program per the farm operating procedures. Cows received AIE if an AEA occurred any time after the previous insemination and before pregnancy diagnosis. Cows not reinseminated and non-pregnant were enrolled in resynchronization protocols according to the ovarian structures present and the body condition score (**BCS**) at non-pregnancy diagnosis. Cows with a CL were

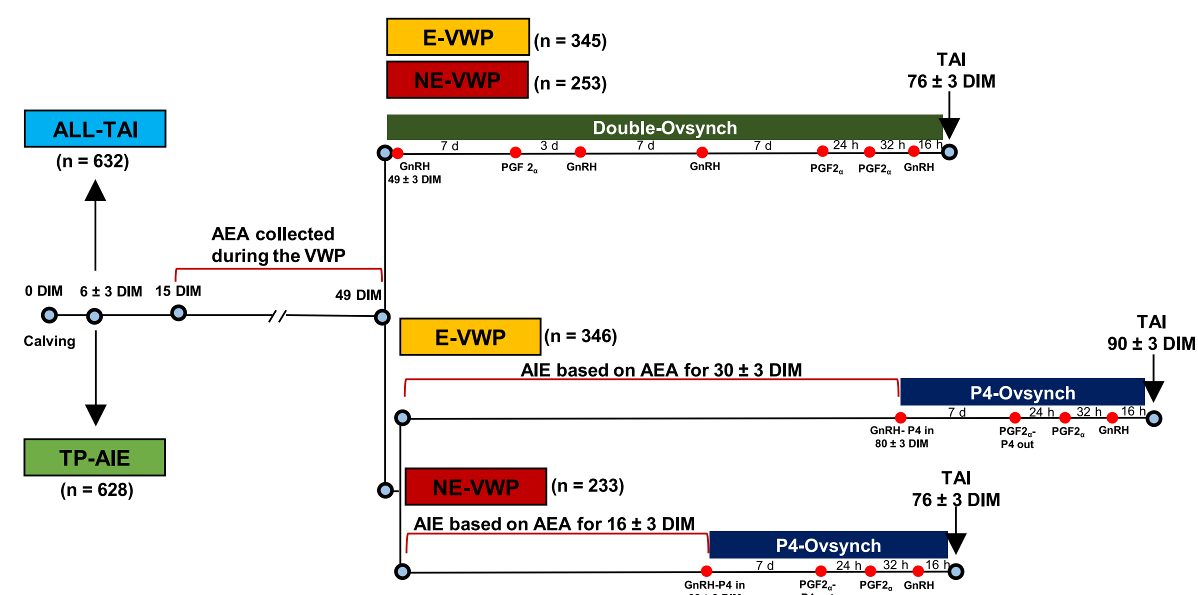

Fig. 1. Graphical depiction of the experimental procedures. Cows fitted with a neck behavior monitoring sensor tag were randomly assigned at 6 ± 3 DIM to one of two reproductive management programs for first service (**ALL-TAI** treatment: n = 632 and **TP-AIE** treatment: n = 628). All cows had automated estrus alerts (**AEA**) collected from 15 to 49 DIM. At 50 DIM cows were classified in estrus groups (**E-groups**). Cows were included in the **E-VWP group** (n = 691; ALL-TAI: n = 345, TP-AIE: n = 346) if had at least one AEA recorded from 15 to 49 DIM and were included in the **NE-VWP group** (n = 486; ALL-TAI: n = 253, TP-AIE: n = 233) if no AEA was recorded from 15 to 49 DIM. Cows assigned to the **ALL-TAI** treatment received a Double-Ovsynch protocol with two $PGF_{2\alpha}$ treatments and received TAI at 76 ± 3 DIM. Cows assigned to the targeted predominant AIE treatment (**TP-AIE**) were eligible to receive AI at detected estrus (**AIE**) for 30 ± 3 or 16 ± 3 DIM after a 49 d VWP if in the E-VWP or if in the NE-VWP group, respectively. Cows that did not receive AIE were submitted to an Ovsynch protocol with progesterone (**P4**) supplementation (**P4-Ovsynch**) and received TAI at 90 ± 3 DIM and 76 ± 3 DIM for E-VWP and NE-VWP group, respectively.

enrolled in a Short-Resynch protocol with two $PGF_{2\alpha}$ and an estradiol cypionate (**ECP**) treatment ($PGF_{2\alpha}$, 1 d later $PGF_{2\alpha}$ and ECP, 32h later GnRH, 16 h later TAI). Cows without a CL, only follicular structures present, and BCS >2.5 were enrolled in an Ovsynch protocol with two $PGF_{2\alpha}$ treatments and an ECP treatment (GnRH, 7 d later $PGF_{2\alpha}$, 24 h later $PGF_{2\alpha}$ and ECP, 32 h later GnRH, 16 h later TAI). Finally, cows without a CL, only follicular structures present, and BCS ≤2.5 or with a putative cyst (≥1 follicle >25 mm) were submitted to an Ovsynch protocol with P4 supplementation, two $PGF_{2\alpha}$ treatments, and an ECP treatment (GnRH and intravaginal P4-releasing device, 7 d later $PGF_{2\alpha}$ and ECP and P4-releasing device removal, 24 h later $PGF_{2\alpha}$, 32 h later GnRH, and 16 h later TAI). All ECP treatments consisted of an i.m. injection of 1 mg estradiol cypionate (E.C.P, Zoetis, Ciudad de Mexico, Mexico).

2.4. Covariate data collection

Calving events from April through September were considered to occur during the warm season whereas events from October through March were considered to occur during the cold season. Calving ease score was recorded on a scale of 1–3 by farm personnel; 1 = unassisted delivery, 2 = low to moderate assistance, and 3 = complex assistance (i.e., mechanical assistance, C-section surgery).

The predicted transmitting abilities based on pedigree estimations for Daughter Pregnancy Rate (**DPR**) were available for all cows enrolled in the study. Three DPR groups were created for primiparous (tertile limits = −1.1 and 0.0) and multiparous cows (tertile limits = −1.4 and = −0.6) based on DPR tertiles.

All health disorders diagnosed by farm personnel from parturition to the end of the VWP were recorded. Every day at 0800 h and 1400 h, cows were selected for clinical examination after milking based on alerts (i.e., Health Index ≤ 86) and rumination data from the automated behavior monitoring system complemented with visual observation of clinical signs of disease. Milkers monitored all cows for clinical signs of mastitis during each milking session. Health events considered for analysis were milk fever, retained placenta, metritis, clinical mastitis, displaced abomasum, indigestion, pneumonia, and lameness [locomotion score (**LS**) ≥ 3 vs. < 3; 1 = normal, 2 = mildly lame, 3 = moderately lame, 4 = lame, 5 = severely lame [10]. Cows were classified in health event groups (i.e., yes or no health events) based on the presence or absence of at least one health event recorded between parturition and 14 DIM or between 15 and 49 DIM. These periods correspond to the time before and during (i.e., 15 to 49 DIM) which AEA were recorded to classify cows in the E-VWP or NE-VWP groups.

Inseminations from April through September were considered to occur during the warm season and inseminations from October through March considered to occur during the cold season.

2.5. Physiological data collection

A body condition score on a scale of 1–5 with 0.25-point increments [11] was assigned to all cows upon entry in the close-up pen, at parturition, at 6 ± 3, 34 ± 3, and 46 ± 3 DIM, and at first AI. Scores were assigned by farm personnel and members of the research team trained to use similar criteria. The BCS change between calving and 46 ± 3 DIM was calculated [12] to group cows based on BCS change (Δ): gain (ΔBCS >0), no change (ΔBCS = 0), and loss of BCS (ΔBCS <0). Locomotion scores were recorded at 34 ± 3 and 46 ± 3 DIM for all cows, as previously described. A LS ≥ 3 in at least one of the two time-points evaluated was considered lameness.

In a subset of cows (n = 343) enrolled from July to October 2021 in the ALL-TAI (n = 168) and TP-AIE (n = 175) treatments, blood samples, and vaginal discharge scores (**VDS**) were collected at 34 ± 3 and 46 ± 3 DIM. Blood samples were collected by puncture of the coccygeal vein or artery using 8-mL sodium heparinized evacuated tubes (BD Vacutainer; Becton Dickinson and Co., Franklin Lakes, New Jersey, USA) to determine circulating concentrations of P4. Samples were placed on ice after collection and centrifuged at 3000×*g* for 20 min at 4 °C. Plasma was collected and stored in 2 mL Eppendorf tubes at −20 °C until sample analysis. Plasma P4 concentrations were determined in single samples using a commercially available solid-phase, no extraction RIA (ImmuChem Coated Tube; MP Biomedicals, Solon, Ohio, USA). Plasma samples with known high (5.3 ng/mL) and low (1.1 ng/mL) concentrations of P4 were included at the beginning and the end of each assay (n = 8) to assess reliability. Average intra- and inter-assay coefficient of variation for the high-concentration sample was 7.8 % and 6.8 %, respectively, and 8.5 % and 11.0 %, respectively, for the low-concentration sample. The detection limit of the assay was 0.1 ng/mL. The presence of a functional CL at 34 ± 3 and 46 ± 3 DIM was defined as P4 ≥1.0 ng/mL. Cows were considered 'cyclic' if P4 ≥ 1 ng/mL in at least one of the plasma samples collected during the VWP. The vaginal discharge was evaluated using a Metricheck device (Metricheck, Simcro, Lamone, Switzerland) and VDS were assigned using the following criteria: 1 = clear or translucent mucus, 2 = mucus with flecks of white or nearly white pus, 3 = discharge with less than 50 % of purulent material, 4 = discharge with 50 % or greater purulent material, 5 = score 4 with stench (Kawashima et al., 2018). A cow was considered to have purulent vaginal discharge (**PVD**) if VDS ≥ 3 at least once during the VWP.

2.6. Statistical analysis

Unless otherwise stated, all statistical analyses were conducted using SAS software (version 9.4, SAS Institute Inc., Cary, North Carolina, USA).

2.6.1. Sample size calculation

A sample size calculation was performed using the survival (time to event) option of the sample size calculation menu of WinPepi version 11.65 (Abramson, 2016). A total of 455 cows per treatment were required to detect a significant difference for a one-tailed test with α of 0.05 and β of 0.20, expected hazard ratio (**HR**) for time to pregnancy of 1.25, and average proportion of cows pregnant at 150 DIM of 0.70. To compensate for cows that left the herd before 150 DIM, ~25 % more cows were included.

2.6.2. Cow removal

Criteria for removal from statistical analyses included lack of outcomes of interest due to death, sale, no eligibility for pregnancy, malfunctioning or missing sensors, insemination errors, diagnosis of endometritis during synchronization of ovulation, and failure to comply with synchronization of ovulation protocols.

2.6.3. Performance outcomes of interest

Binary outcomes included the proportion of cows with one or more AEA during the VWP, the proportion of cows AIE for first and second service, P/AI for first and second service, pregnancy loss, and the proportion of cows pregnant at 150 DIM for the cows that completed the VWP. Physiological binary outcomes evaluated were the proportion of cows with circulating concentrations of P4 ≥ 1 ng/mL and cyclic cows during the VWP. Continuous outcomes evaluated were DIM at first and second AEA during the VWP, DIM at first and second service, and BCS. Count data evaluated were the number of services by 150 DIM. Time to event data evaluated were days to pregnancy by 150 DIM.

Binary outcomes were analyzed using logistic regression with PROC GLIMMIX fitting a binomial distribution. Continuous outcomes were analyzed by ANOVA using PROC MIXED. Assumptions of normality of residuals, linear relationship, and homoscedasticity of variance for residuals in linear regression models were assessed by evaluating normal probability plots and plots of residuals versus predicted values. Due to the lack of normality of the distribution of data for DIM at the second AEA during the VWP (negative skewness), data were squared and then back transformed for reporting. The number of services by 150 DIM for cows that completed the 150 d experimental period was analyzed using logistic regression with PROC GLIMMIX fitting a Poisson distribution. Time to pregnancy up to 150 DIM was analyzed using Cox proportional hazard regression models with PROC PHREG for all cows that completed the VWP. Days to pregnancy and survival curves were obtained using the Kaplan-Meier survival analysis of the MedCalc Software (version 22.009, MedCalc Software Ltd., Ostend, Belgium).

Logistic and linear regression models for analysis of the AEA dynamics during the VWP included treatment and parity (primiparous vs. multiparous) as main fixed effects. Season of parturition, calving ease score, health disorder occurrence group by 14 DIM, DPR group, and all 2-way interactions with treatment were offered as covariates. Model adjustments were needed due to experimental design constraints and the nature of some data. Models used to evaluate DIM at first AEA during the VWP were offered number of AEA during the VWP (1 vs. > 1) and the interaction with treatment as covariates. Models to evaluate the insemination dynamics (DIM at first and second AI, and reinsemination at detected estrus) were offered estrus-group (E-group), health disorder occurrence from 15 to 49 DIM, and the 2-way interactions with treatment as covariates. The proportion of cows AIE for first service for cows in the TP-AIE treatment only was analyzed with a model including E-group as the main fixed effect and all 2-way interactions between the fixed effects treatment or grouping variable and covariates offered to the initial models.

Data for BCS from the time of parturition to the end of the VWP were analyzed using ANOVA with repeated measures and included time, treatment, parity, and E-group as fixed effects. Cows were the subject of repeated measurements. The Toeplitz covariance structure was used as it minimized the Akaike Information Criterion. Body condition score on the day of first service was analyzed by ANOVA and included parity, treatment, E-group, and all 2-way interactions as fixed effects. The association between BCS change from calving to the end of VWP, PVD, and lameness with estrus expression (i.e., E-group) during the VWP were evaluated using logistic regression models. Parity was included as a fixed effect and the interactions between BCS, PVD, or lameness with parity were offered to the models.

The proportion of cows with circulating concentrations of P4 $\geq$ 1 ng/mL at 34 and 46 DIM and the proportion of cows considered cyclic during the VWP was compared by E-group and treatment using logistic regression models in PROC GLIMMIX fitting a binomial distribution. Models included treatment, E-group, and parity as main fixed effects. The interaction between treatment and E-group was also offered. The percentage of cyclic and non-cyclic cows with AEA during the VWP were calculated using the FREQ procedure.

Models for P/AI and pregnancy loss included the main fixed effects of treatment, parity, E-group, and the interaction between treatment and E-group. Season of AI, health disorder occurrence up to 14 DIM and from 15 to 49 DIM, DPR group, and all possible 2-way interactions with treatment were offered as covariates. For the proportion of cows pregnant and time to pregnancy by 150 DIM, season of parturition was offered as covariate.

The final model for each outcome of interest was selected by manual backward elimination of covariates with $P > 0.10$. Explanatory variables and relevant interactions were considered significant if $P \leq 0.05$ and tendencies if $0.05 < P \leq 0.10$. When appropriate, the least significant difference (**LSD**) post hoc mean separation test was used to determine differences between least squares means (**LSM**). Results are reported as LSM [95 % confidence interval (**CI**)] for binary outcomes and LSM $\pm$ standard error of the mean for continuous outcomes.

3. Results

3.1. Study population

In total, 38.6 % of the cows enrolled (n = 1260) were primiparous and 61.4 % were multiparous, 35.7 % calved during the cold and 64.3 % during the warm season. Out of all cows, 87.9 % had a calving ease score of 1, 7.4 % had a score of 2, and 4.8 % had a score of 3. Out of all AI services, 52.0 % occurred during the warm and 48.0 % during the cold season. The mean PTA for DPR groups were -2.1 ± 0.6, -0.9 ± 0.4, and 0.4 ± 0.6 for the low, medium, and high DPR group, respectively. At least one health disorder was recorded within the first 14 DIM for 18.7 % of the cows and at least one event was recorded from 15 to 49 DIM in 6.3 % of the cows.

Cows were removed from analyses because of automated estrus detection system malfunction (0.2 %), lack of compliance with experimental protocols (1.1 %) and delayed insemination due to endometritis (1.7 %). In total, 18.5 % of the cows left the herd due to death (1.6 %), sale (15.5 %) or were coded as do not breed (1.3 %). Out of the 1260 cows enrolled, 1177 (93.4 %) completed the VWP, 1097 received first service (87.1 %), 1074 (85.2 %) and 1066 (84.6 %) underwent a first and second pregnancy diagnosis, respectively, and 988 (78.4 %) completed the experimental period of 150 DIM.

The percentage of AEA recorded during the VWP and used to include cows in the E-VWP or NE-VWP and the reasons for exclusion are presented in Supplemental Table S1. Out of all AEA recorded from calving until the end of the VWP, 38.9 % were not considered true estrus alerts and were ignored for the classification of cows in the E-VWP and NE-VWP group. Out of the alerts ignored, 20.0 % were due to the AEA occurring before 15 DIM, 10.6 % because the AEA was observed on the day of or after a pen move, and 8.3 % because the cow was confirmed not in estrus by farm personnel.

3.2. Dynamics of AEA recorded during the VWP and associations with physiological outcomes

Results for the percentage of cows with AEA during the VWP are reported in Table 1. There was an interaction between treatment and health disorder occurrence before 14 DIM on expression of estrus during the VWP ($P = 0.04$) as there were differences for cows with and without health disorders within treatment but there was no difference between the group with or without health disorders across the treatments. More cows had AEA during the VWP if calved during the cold than the warm season ($P = 0.04$), if in the high than the low and medium DPR groups ($P < 0.001$), and there was a tendency for more primiparous than multiparous cows ($P = 0.06$) to have AEA.

Out of the 691 cows in the E-VWP group, 78.5 % had 1 AEA, 21.1 % had 2 AEA, and 0.4 % of the cows had 3 AEA. There was no difference in the percentage of cows that presented >1 AEA between treatments (ALL-TAI = 19.5 % [14.4, 25.9] and TP-AIE = 15.3 % [10.9, 21.2], $P = 0.12$) or parity groups (primiparous = 19.4 % [14.0, 26.2] and multiparous = 15.5 % [11.2, 21.0], $P = 0.14$). More cows without (23.1 % [19.9, 26.7]) than with ≥1 health disorder (12.8 % [7.2, 21.7]) by 14 DIM had ≥1 AEA during the VWP ($P = 0.03$).

There was a tendency for an interaction between treatment and health disorder occurrence by 14 DIM on time to the first AEA ($P = 0.08$). Cows with health disorders in the ALL-TAI treatment tended to have the first AEA earlier (26.6 ± 0.7 DIM) than cows in the TP-AIE treatment with (30.8 ± 1.4 DIM) or without health disorders (29.8 ± 0.5 DIM). Conversely, cows in the ALL-TAI treatment without health events did not differ from any group (28.9 ± 0.5 DIM). The first AEA was observed earlier for multiparous (27.8 ± 0.6 DIM, $P < 0.001$) than primiparous (30.3 ± 0.6 DIM) cows, and for cows with more than one (23.0 ± 0.8 DIM, $P < 0.001$) than only one AEA (35.1 ± 0.5 DIM). Days at the second AEA (n = 149) did not differ between treatments (42 ± 0.7 and 41.7 ± 0.7 DIM for the ALL-TAI and TP-AIE treatment, respectively; $P = 0.25$) or parity ($P = 0.83$).

The percentage of cows with an AEA during the VWP for the subset of cows included in the physiological data analysis was consistent with the observations for all cows and did not differ by treatment (ALL-TAI = 60.0 % [52.3, 67.2] and TP-AIE = 60.8 % [53.2, 67.9]; $P = 0.90$]). Out of the cows with physiological data, 23.0 % were considered to have PVD.

Table 1
Percentage of cows with automated estrus alerts during the voluntary waiting period for cows enrolled at 6 ± 3 DIM to (1) a Double-Ovsynch protocol for TAI at 76 ± 3 DIM (**ALL-TAI**) or (2) a targeted reproductive management program that prioritized AI at detected estrus (**TP-AIE**). Data are presented as unadjusted means (%, n/n) and least square means [95 % CI].

Variable	Level	n	Unadjusted mean %, (n)	LSM [95 % CI], %	*P*-value
Treatment	ALL-TAI	598	57.7 (345)	56.1 [50.6, 61.5]	0.41
	TP-AIE	579	59.8 (346)	52.8 [46.8, 58.7]	
Parity	Primiparous	453	61.8 (280)	57.4 [51.8, 62.8]	0.06
	Multiparous	724	56.8 (411)	51.5 [46.8, 56.2]	
Season of parturition[a]	Cold	424	63.2 (268)	57.8 [52.1, 63.3]	0.04
	Warm	753	56.2 (423)	51.1 [46.5, 55.7]	
DPR Tertile[b]	Low	344	50.6 (174)	45.9[b] [39.8, 52.0]	<0.001
	Medium	471	56.5 (266)	52.2[b] [46.8, 57.5]	
	High	362	69.3 (251)	64.9[a] [58.8, 70.4]	
Health disorder before 14 DIM[c]	Yes	198	42.9 (85)	44.8 [37.7, 52.1]	<0.001
	No	979	61.9 (606)	63.8 [60.4, 67.0]	
Treatment × health disorder before 14 DIM	ALL-TAI × Yes	105	47.6 (50)	50.6[b] [40.8, 60.3]	0.04
	ALL-TAI × No	493	59.8 (295)	61.4[a] [56.8, 65.8]	
	TP-AIE × Yes	93	37.6 (35)	39.1[b] [29.5, 49.7]	
	TP-AIE × No	486	64.0 (311)	66.0[a] [61.5, 70.3]	

a-b means for levels of a variable with different superscripts differ ($P \leq 0.05$) based on the LSD post hoc mean separation test.
[a] Season of parturition = birth events occurred from April to September were considered to occur during the warm season whereas birth events from October to March during the cold season.
[b] DPR tertiles were created for primiparous (medium tertile lower limit = −1.1 and upper limit = 0.0) and multiparous cows (medium tertile lower limit = −1.4 and upper limit = −0.6).
[c] Cows with (Yes) or without (No) health disorders diagnosed between parturition and 14 DIM.

More cows without (64.6 % [58.5, 70.3]) than with PVD (45.4 % [34.7, 56.5], $P = 0.003$) had AEA recorded during the VWP. Out of the cows with LS data (n = 1094), 17.8 % had lameness during the VWP. More ($P < 0.001$) cows without (62.0 % [58.8, 65.1]) than with (44.9 % [37.5, 52.5]) lameness during the VWP had an AEA. For all cows that completed the VWP, there was a treatment by time interaction for BCS ($P < 0.001$) because the mean BCS was greater at 6 ± 3 DIM for the ALL-TAI treatment (ALL-TAI = 3.25 ± 0.01 and TP-AIE = 3.20 ± 0.01, Supplemental Fig. 1A). Also, cows in the E-VWP group had greater BCS than cows in the NE-VWP group from 6 ± 3 DIM until 46 ± 3 DIM ($P < 0.001$, Supplemental Fig. 1B). Greater BCS was also observed for primiparous cows ($P < 0.001$), cows without a health disorder through 14 DIM ($P = 0.005$), and there was a tendency for greater BCS for cows that calved in the cold season ($P = 0.06$). Out of the cows with BCS measures at calving and the end of the VWP (n = 988), 84.2 % lost (0.60 ± 0.28), 12.9 % (no BCS variation) maintained, and 2.9 % gained (0.30 ± 0.15) BCS, respectively. Mean BCS at calving and 46 ± 3 DIM were 3.5 ± 0.3 and 2.9 ± 0.3 for cows that lost, 3.33 ± 0.3 and 3.33 ± 0.3 for cows that maintained, and 3.1 ± 0.3 and 3.4 ± 0.3 for cows that gained BCS. Cows that maintained BCS were more likely to have an AEA during the VWP

Table 2
Percentage of cows with circulating concentrations of progesterone (P4) ≥ 1 ng/mL at 34 ± 3 DIM and 46 ± 3 DIM, and percentage of cyclic cows by the end of the VWP. Cows were defined as cyclic if plasma P4 concentrations ≥1 ng/mL in at least one of the two time-points evaluated. Results of the effect of the treatment, E-group and parity on the mentioned outcomes are reported. Data are presented in least squares means [95 % CI] and unadjusted means (%, n/n).

Outcome and variable	Level	N	Unadjusted mean %, (n)	LSM [95 % CI], %	*P*-value
P4 > 1 ng/mL at 34 ± 3 DIM		343			
E-group[a]	E-VWP[b]	205	62.4 (128)	62.0 [55.0, 68.4]	0.001
	NE-VWP[c]	138	44.9 (62)	43.7 [35.5, 52.3]	
Treatment[d]	ALL-TAI[e]	168	52.4 (88)	49.7 [41.9, 57.6]	0.25
	TP-AIE[f]	175	58.3 (102)	56.1 [48.4, 63.6]	
Parity	Primiparous	147	49.7 (73)	47.3 [39.0, 55.6]	0.04
	Multiparous	196	59.7 (117)	58.6 [51.3, 65.4]	
P4 > 1 ng/mL at 46 ± 3 DIM		343			
E-group	E-VWP	205	70.7 (145)	70.4 [63.7, 76.3]	<0.001
	NE-VWP	138	48.6 (67)	47.2 [38.8, 55.7]	
Treatment	ALL-TAI	168	60.7 (102)	58.0 [50.0, 65.7]	0.65
	TP-AIE	175	62.9 (110)	60.5 [52.7, 67.9]	
Parity	Primiparous	147	55.1 (81)	52.3 [43.9, 60.7]	0.01
	Multiparous	196	66.8 (131)	65.9 [58.7, 72.4]	
Cyclic, %		343			
E-group	E-VWP	205	93.2 (191)	93.5 [89.2, 96.2]	<0.001
	NE-VWP	138	60.1 (83)	58.3 [49.5, 66.6]	
Treatment	ALL-TAI	168	78.0 (131)	79.6 [71.7, 85.8]	0.35
	TP-AIE	175	81.7 (143)	83.9 [76.8, 89.1]	
Parity	Primiparous	147	72.8 (107)	72.8 [63.6, 80.4]	<0.001
	Multiparous	196	85.2 (167)	88.3 [82.6, 92.4]	

[a] E-group = cows grouped based on detection of estrus (yes vs. no) during the VWP (E-VWP vs. NE-VWP, respectively).
[b] E-VWP = cows with at least one estrus event based on automated estrus alerts (AEA) recorded during the VWP.
[c] NE-VWP = cows without an estrus event based on AEA recorded during the VWP.
[d] Treatment = treatment used for first service.
[e] ALL-TAI = cows received a Double-Ovsynch protocol with TAI at 76 ± 3 DIM.
[f] TP-AIE = cows received AI at detected estrus (AIE), if detected in estrus, for 30 ± 3 or 16 ± 3 d after a 49 d VWP. Cows not AIE with or without AEA during the VWP received TAI after P4-Ov at 90 ± 3 or 76 ± 3 DIM, respectively.

(72.1 % [63.6, 79.2], $P = 0.01$) than cows that lost (58.3 [54.8, 61.7] or gained BCS (53.5 % [35.6, 70.6]).

The proportion of cyclic cows by the end of the VWP as determined by evaluation of P4 concentrations 12 d apart at 34 and 46 DIM was 79.9 %. More cows in the E-VWP than the NE-VWP group had plasma P4 concentrations ≥1 ng/mL at 34 ($P = 0.001$) and 46 DIM ($P < 0.001$), and more cows were cyclic in the E-VWP than the NE-VWP group by the end of the VWP (Table 2). There was no effect of treatment ($P > 0.20$; Table 2) but fewer primiparous than multiparous cows had plasma P4 concentrations ≥1 ng/mL at all time-points ($P < 0.05$; Table 2).

On the other hand, the proportion of cyclic cows with AEA during the VWP was 69.7 % and the proportion of non-cyclic cows with AEA was 20.3 %.

3.3. Insemination dynamics

3.3.1. First service

Cows in the TP-AIE treatment and E-VWP group (67.8 ± 0.8 DIM) had first service earlier ($P = 0.009$) than cows in the TP-AIE and NE-VWP group (70.2 ± 0.8 DIM), whereas no difference was observed for cows in the ALL-TAI treatment (E-VWP ALL-TAI = 75.7 ± 0.8 DIM and NE-VWP ALL-TAI = 75.7 ± 0.9 DIM). There was also an interaction between treatment and health disorder group from 15 through 49 DIM ($P = 0.04$), such that cows with (75.5 ± 1.5 DIM) and without (75.9 ± 0.3 DIM) a health disorder in the ALL-TAI treatment had first service at later DIM than cows in the TP-AIE treatment. Cows without health disorders were inseminated earlier (67.0 ± 0.3 DIM) than cows with ≥1 health disorder (71.0 ± 1.4 DIM). Overall, cows in the TP-AIE treatment (69.0 ± 0.7 DIM) were inseminated earlier than cows in the ALL-TAI treatment (75.7 ± 0.8 DIM) for AIE and TAI services combined. Primiparous cows (72.0 ± 0.6 DIM) tended to be inseminated earlier than multiparous cows (72.8 ± 0.6, $P = 0.10$).

The proportion of cows AIE in the TP-AIE treatment was 69.3 % (n = 365/527), and there was an interaction between E-group and health disorder by 14 DIM ($P = 0.03$; data not shown) because more of the NE-VWP group cows with health disorders received AIE than the NE-WVP group cows without health disorders. The opposite was observed for the E-VWP group. Overall, a greater percentage of cows in the E-VWP group (83.3 % [76.3, 88.6]) than in the NE-VWP group (45.0 % [36.9, 53.3]) received AIE ($P < 0.001$) and there was a tendency ($P = 0.09$) for more primiparous (71.0 % [62.4, 78.3]) than multiparous cows (62.5 % [54.8, 69.7]) to receive AIE.

Days in milk at first AI for cows in the TP-AIE group (n = 527) was affected by the interaction between E-group and the type of service (AIE vs. TAI; $P < 0.001$). Cows received AIE earlier in the NE-VWP (58.6 ± 0.8 DIM) than the E-VWP group (63.4 ± 0.6 DIM) and received TAI earlier in the NE-VWP (76.5 ± 0.7 DIM) than the E-VWP group (91.3 ± 1.0 DIM). There was also an effect (results not shown) of health disorders from 15 to 49 DIM ($P = 0.04$), the interaction between E-group and calving season ($P = 0.02$), and there tended to be an interaction between E-group and parity ($P = 0.06$).

There was a tendency for an interaction between treatment and health disorders by 14 DIM ($P = 0.09$) for BCS at first service because within the ALL-TAI treatment, cows with health disorders had lower BCS (2.86 ± 0.03) than cows without health disorders (2.94 ± 0.01), whereas no difference was observed within the TP-AIE (2.98 ± 0.03). Overall, BCS was greater ($P < 0.05$) for cows in the TP-AIE (2.98 ± 0.01) than the ALL-TAI treatment (2.91 ± 0.02), and cows in the E-VWP (2.98 ± 0.02) than the NE-VWP group (2.90 ± 0.02). Greater BCS was also observed for primiparous than multiparous cows ($P < 0.001$), cows that calved in the cold season ($P = 0.02$), and cows in the high than in the medium and low DPR groups ($P = 0.04$).

3.3.2. Second service

There was a treatment by calving season interaction ($P = 0.04$), such that fewer cows in the ALL-TAI treatment that calved during the cold season were reinseminated at detected estrus (53.3 % [39.7, 66.4]) than cows in the TP-AIE treatment that calved during the cold season (68.3 % [55.5, 78.7]) and cows in the ALL-TAI treatment that calved during the warm season (66.1 % [55.2, 75.5]). Cows in the TP-AIE treatment that calved during the warm season did not differ from any group (62.6 % [52.1, 72.0]; $P > 0.10$). In addition, there was an interaction between treatment and DPR tertile ($P = 0.008$), such that cows on the ALL-TAI treatment and high tertile for DPR (72.8 % [58.7, 83.5]) and cows in the high (62.4 % [48.6, 74.5]), medium (69.4 % [57.5, 79.2]) and low (64.4 % [51.3, 75.7]) DPR tertile for the TP-AIE treatment had a greater percentage of cows reinseminated at detected estrus than cows on the ALL-TAI treatment in the medium tertile (46.9 % [35.0, 59.4]). Cows in the low tertile for DPR that received the ALL-TAI treatment (58.2 % [44.1, 71.1]) had a similar proportion of cows reinseminated at detected estrus compared to the other groups. Overall, there was no significant difference between treatments ($P = 0.21$), but more cows in the E-VWP (74.7 % [66.2, 81.7]) than the NE-VWP group were reinseminated at detected estrus (48.9 % [38.5, 59.4]; $P < 0.001$). Fewer cows with (52.2 % [35.0, 68.9]; $P = 0.02$) than without (72.1 % [67.9, 76.0]) health disorders from 15 to 49 DIM were reinseminated at detected estrus.

There was an interaction between treatment and calving season ($P = 0.03$) for DIM at second service, because regardless of season of parturition, cows were reinseminated earlier in the TP-AIE (cold = 100.1 ± 2.0 and warm = 102.0 ± 1.7) than the ALL-TAI treatment, for which cows that calved during the cold season were reinseminated later (114.6 ± 2.1) than cows that calved during the warm season (110.7 ± 1.7). Overall, DIM reinsemination occurred earlier for cows in the TP-AIE (101.1 ± 1.6) than the ALL-TAI treatment (112.7 ± 1.7; $P < 0.001$), in the E-VWP (103.6 ± 1.6; $P < 0.001$) than the NE-VWP group (110.2 ± 1.7), and if in the no health disorder (104.0 ± 0.7) than the health disorder from 15 to 49 DIM group (109.7 ± 2.8; $P = 0.03$).

3.4. Pregnancies per AI

3.4.1. First service

For P/AI at 34 d after AI for AIE and TAI services combined (n = 1074), there was an interaction between treatment and AEA group during the VWP ($P = 0.03$) because cows in the TP-AIE treatment and NE-VWP group had fewer P/AI than the other groups (Table 3). Also, primiparous cows ($P < 0.001$), cows without health disorders by 14 DIM ($P = 0.01$), and cows in the medium and high DPR group ($P = 0.05$) had more P/AI. Overall, the ALL-TAI treatment (42.3 % [37.1, 47.7]) had more P/AI than the TP-AIE treatment (29.0 % [24.2, 34.3]; $P < 0.001$) and the E-VWP group (42.5 % [37.4, 47.7]) had more P/AI than the NE-VWP group (28.9 % [24.0, 34.3]; $P < 0.001$). The effect of covariates on P/AI are reported in Supplemental Table S2.

For P/AI at 62 d after AI, there was a tendency for an interaction between treatment and E-group during the VWP ($P = 0.06$), and effect of parity ($P < 0.001$) and health disorders by 14 DIM ($P = 0.07$). The direction of the differences among groups was the same as for P/AI at 34 d (Table 3).

For pregnancy loss, there was no effect of treatment ($P = 0.45$) but there was an effect of E-group ($P = 0.01$) because cows in the E-VWP group (9.1 % [5.9, 13.7]; $P = 0.01$) had fewer pregnancy losses than cows in the NE-VWP group (18.6 % [12.5, 26.7]; Table 3). There was no effect of parity affected pregnancy loss ($P = 0.13$; Supplemental Table S2).

3.4.2. Second service

There was an interaction between treatment and parity ($P = 0.05$) such that there was no difference between primiparous cows in the ALL-TAI (56.4 % [46.5, 65.9]) and TP-AIE treatment (44.5 % [36.0, 53.4]) whereas primiparous cows in the ALL-TAI treatment had more P/AI than multiparous cows in both treatments (ALL-TAI = 29.7 % [23.9, 36.2]; TP-AIE = 34.1 % [28.2, 40.6]). Cows in the E-VWP group (46.1 % [40.8, 51.5]) had more P/AI than cows in the NE-VWP group (35.8 % [30.2,

Table 3
Pregnancies per AI and proportion of cows pregnant by 150 DIM for cows (1) submitted to a Double-Ovsynch protocol for TAI at 76 ± 3 DIM (ALL-TAI) or (2) enrolled in a targeted reproductive management program that prioritized AIE (TP-AIE). Results for the effect of treatment, E-group (E-VWP vs. NE-VWP), and the interaction between treatment and E-group are reported for all the outcomes. Data are presented in least squares means [95 % CI] and unadjusted means (%, n/n).

	Treatment				P-value		
	ALL-TAI		TP-AIE				
Item rowhead	E-VWP	NE-VWP	E-VWP	NE-VWP	Trt[a]	E-group	Trt × E-group
P/AI 34 ± 3 d after first AI rowhead	46.1[a] [39.8, 52.5] 49.5 (165/333)	38.7[a] [31.9, 46.0] 39.9 (89/223)	38.9[a] [32.7, 45.5] 43.0 (138/321)	20.7[b] [15.4, 27.3] 21.8 (43/197)	<0.001	<0.001	0.03
P/AI 62 ± 3 d after first AI rowhead	42.6 [36.3, 49.1] 45.0 (149/331)	32.3 [25.9, 39.4] 32.9 (73/222)	35.0 [29.0, 41.5] 37.8 (120/317)	16.7 [11.9, 39.4] 17.3 (34/196)	<0.001	<0.001	0.06
Pregnancy loss first AI rowhead	8.7 [5.2, 14.0] 9.1 (15/164)	17.2 [10.6, 26.6] 17.0 (15/88)	11.0 [6.7, 17.6] 11.1 (15/135)	20.1 [10.7, 34.6] 20.9 (9/43)	0.45	0.01	0.90
P/AI 62 ± 3 d after second AI rowhead	51.0 [43.2, 58.7] 45.8 (82/179)	34.5 [26.4, 43.5] 28.2 (40/142)	41.3 [34.3, 48.6] 36.6 (70/191)	37.2 [29.8, 45.3] 34.0 (53/156)	0.43	<0.001	0.12
Proportion pregnant 150 DIM rowhead	70.2 [62.0, 77.3] 76.5 (263/344)	47.0 [37.9, 56.3] 52.8 (132/250)	64.5 [55.8, 72.4] 71.9 (241/335)	47.1 [37.8, 56.5] 51.9 (110/212)	0.33	<0.001	0.32

a-c means for levels of a variable with different superscripts differ ($P \leq 0.05$) based on the LSD post hoc mean separation test.
[a] Trt = treatment used for first service.

41.8]; $P = 0.01$; Table 3).

3.5. Pregnancy dynamics

3.5.1. Time to pregnancy

The hazard ratio (HR = 1.0 [0.9, 1.2]; ALL-TAI referent) for time to pregnancy up to 150 DIM did not differ between treatments ($P = 0.97$; Fig. 2A). Median days to pregnancy were 102 [98, 113] d and 108 [100, 115] d and mean days to pregnancy were 110.3 ± 1.4 d and 108.9 ± 1.5 d for the ALL-TAI and TP-AIE treatments, respectively. Cows in the E-VWP group had a greater ($P < 0.001$) HR for time to pregnancy (HR = 1.6 [1.4, 1.9]) than cows in the NE-VWP group (Fig. 2B), which resulted in median and mean days to pregnancy of 95 [91, 99] d and 133 [120, 145] d for the E-VWP group and 103.2 ± 1.3 d and 119.3 ± 1.6 d for the NE-VWP group. The hazard of pregnancy was also greater for primiparous than multiparous cows (HR = 1.7 [1.5, 1.9]; $P < 0.001$), cows in the high and medium (reference) DPR (HR high vs. medium = 1.1 [0.9, 1.3]) than the low DPR group (HR high vs. low = 1.5 [1.2, 1.8] and HR low vs. medium = 0.8 [0.6, 0.9]), and for cows without than with clinical health disorders through 14 DIM (HR = 1.3 [1.0, 1.6]; $P = 0.02$).

For cows in the ALL-TAI treatment only, the HR for time to pregnancy (HR = 1.6 [1.3, 2.0]) was greater ($P < 0.001$; Fig. 3A) for cows with estrus during the VWP. Median days to pregnancy were 96 [79, 99] d and 137 [119, 146] d and mean days to pregnancy were 104.3 ± 1.7 d and 119.2 ± 2.2 d for cows with and without estrus, respectively. The HR for time to pregnancy was also greater (HR = 1.8 [1.5, 2.2]; $P < 0.001$) for primiparous than multiparous cows and tended to differ by DPR tertile group ($P = 0.08$) as observed for the analysis of all cows combined.

For cows in the TP-AIE treatment only, the HR for time to pregnancy was greater (HR = 1.6 [1.3, 2.1]) for cows with an AEA during the VWP ($P < 0.001$; Fig. 3B). Accordingly, median and mean days to pregnancy were 93 [89, 103] d and 102.0 ± 2.0 d for cows with an AEA and 126 [116, 150] d and 119.6 ± 2.3 d for cows with no AEA. The effects of covariates were similar than for the analysis of all cows combined.

3.5.2. Cows pregnant by 150 DIM

There was no effect of treatment (ALL-TAI = 59.1 % [51.2, 66.6] vs. TP-AIE = 56.0 % [48.0, 63.7]; $P = 0.33$) or the interaction between treatment and E-group ($P = 0.32$), whereas more cows were pregnant by 150 DIM in the group with (67.4 % [60.1, 74.1]; $P < 0.001$) than without an AEA during the VWP (47.0 % [39.1, 55.1]). Also, more cows in the primiparous than multiparous group ($P < 0.001$), more cows in the high than the medium and low DPR groups ($P < 0.001$), and more cows without than with health disorder events by 14 DIM ($P = 0.006$) and between 15 and 49 DIM ($P = 0.05$) were pregnant by 150 DIM (Supplemental Table S2). For this analysis, cows not in compliance with experimental protocols (n = 14; ALL-TAI: n = 1; TP-AIE: n = 13) and delayed insemination due to endometritis (n = 22; ALL-TAI: n = 3; TP-AIE: n = 19) were not considered.

3.5.3. Number of AI services

The average number of AI services received was greater for the TP-AIE (2.10 ± 0.06) than the ALL-TAI treatment (1.82 ± 0.06; $P = 0.001$), and for multiparous than primiparous cows ($P < 0.001$). Similarly, cows without an AEA during the VWP (NE-VWP = 2.04 ± 0.07) had more AI services than cows with an AEA (E-VWP = 1.86 ± 0. 05; $P = 0.03$). There was no interaction between treatment and E-group ($P = 0.95$). The frequency distribution of the number of services received by 150 DIM are reported in Table 4.

4. Discussion

In support of our primary hypothesis, the TRM program designed to optimize use of AIE and TAI based on automated estrus alerts during the VWP resulted in a similar hazard ratio for time to pregnancy and a similar proportion of cows pregnant by 150 DIM compared with a fertility program that used all-TAI after a Double-Ovsynch protocol and had an extended VWP (i.e., 76 ± 3 DIM). The low overall P/AI (21 %) for cows in the TP-AIE treatment that did not have estrus alerts during the VWP was compensated for by almost double the P/AI in cows that had estrus alerts during the VWP. Pregnancies per AI in the range of 40 % for more than half of the cows and a 26 d shorter VWP were critical factors to the success of the TP-AIE program. Like previous experiments in which programs for first service management had different VWP durations [3,13,14], earlier reinsemination of cows not pregnant to first service led to a similar pregnancy dynamic for both treatments. In the case of programs that combine AIE and TAI with a shorter VWP, such as the TP-AIE treatment in this experiment, not only are cows reinseminated earlier but also have more opportunities to conceive per unit of time as these cows are inseminated more times than when managed with programs that enhance fertility through all-TAI and extended VWP. This helps compensate for the fewer first service P/AI typically observed when programs that combine AIE and TAI are compared with all-TAI programs after Double-Ovsynch and an extended VWP [3]. Indeed, by the time cows received first service in the ALL-TAI treatment, 15 % of the cows in the TP-AIE treatment were already reinseminated (90 % received second AI and 10 % third AI). Moreover, by the time cows in the ALL-TAI treatment began to return to estrus and could be reinseminated (i.e., ~90 to 100 DIM), enough pregnancies were created by the TP-AIE treatment as to have the same percentage of cows pregnant as the ALL-TAI treatment.

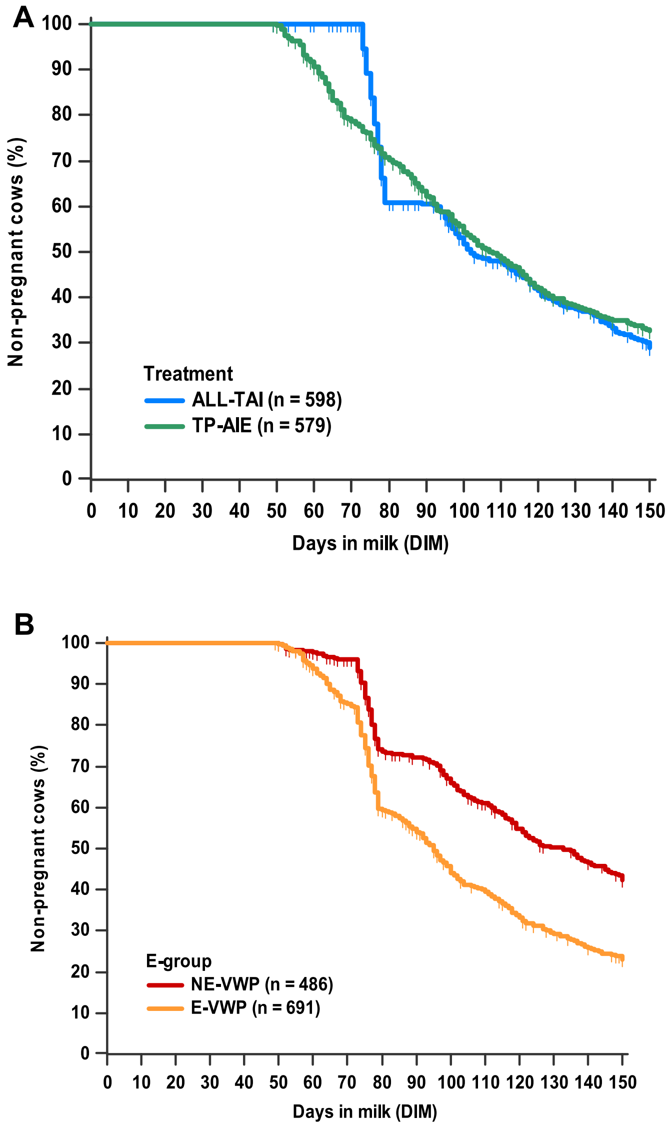

Fig. 2. (A) Survival curve for time to pregnancy by 150 DIM for first service for cows submitted (1) to a Double-Ovsynch protocol for 76 ± 3 DIM (**ALL-TAI**) or (2) to a targeted reproductive management program that prioritized AI at detected estrus (**TP-AIE**). The hazard ratio (**HR**) for time to pregnancy by 150 DIM did not differ between treatments when using the ALL-TAI treatment as referent (HR = 1.0 [0.9, 1.2]; $P = 0.97$). Mean and median days to pregnancy were 110.3 ± 1.4 d and 108.9 ± 1.5 d, and 102 [98, 113] d and 108 [100, 115] d for the ALL-TAI and TP-AIE treatments, respectively. (B) Survival curve for time to pregnancy by 150 DIM for cows with (**E-VWP**) or without (**NE-VWP**) automated estrus alerts (**AEA**) during the VWP. Cows in the E-VWP group had a greater ($P < 0.001$) hazard of pregnancy (HR = 1.6 [1.4, 1.9]) than cows in the NE-VWP with mean and median days to pregnancy of 103.1 ± 1.3 d and 119.2 ± 1.6 d, and 95 [91, 99] d and 133 [120, 145] d, respectively.

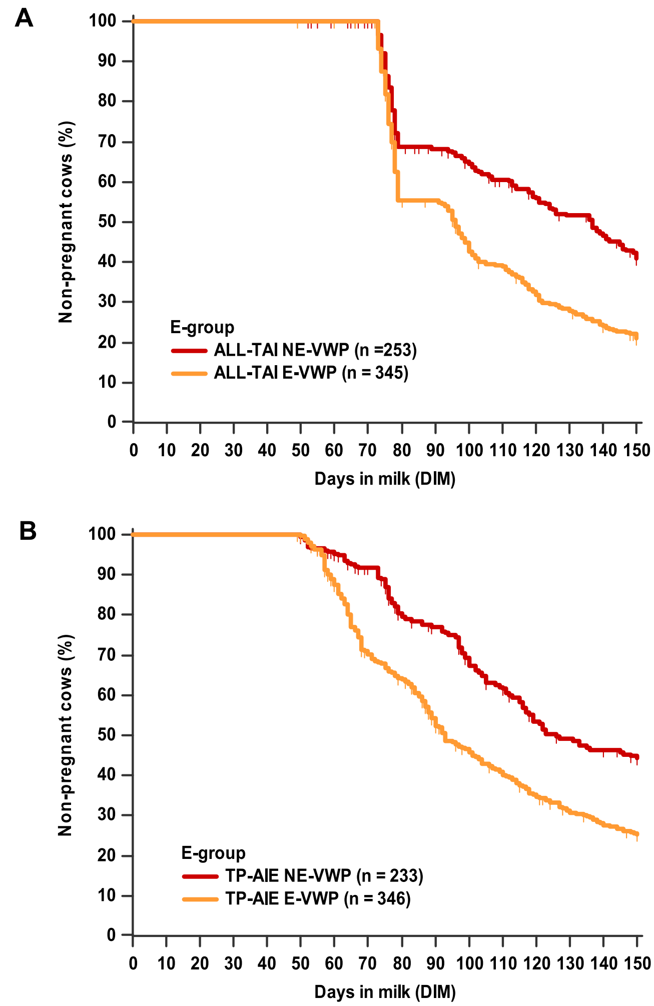

Fig. 3. (**A**) Survival curves for time to pregnancy by 150 DIM for cows with at least one automated estrus alert (**AEA**; **E-VWP group**) or no AEA (**NE-VWP group**) recorded from 15 to 49 DIM that received a Double-Ovsynch protocol for TAI at 76 ± 3 DIM (**ALL-TAI** treatment). The hazard ratio (**HR**) for time to pregnancy was greater (HR = 1.6 [1.3, 2.0]) $P < 0.001$) for cows in the E-VWP than the NE-VWP group. Median days to pregnancy were 96 [79, 99] d and 137 [119, 146] d and mean days to pregnancy were 104.3 ± 1.7 d and 119.2 ± 2.2 d for cows with and without an AEA. (**B**) Survival curves for time to pregnancy to 150 DIM for cows with at least one AEA (**E-VWP group**) or no AEA (**NE-VWP group**) recorded from 15 to 49 DIM submitted to a targeted reproductive management program that prioritized AI at detected estrus (**AIE**; **TP-AIE**) to receive first service. Cows in the E-VWP group had a greater ($P < 0.001$) hazard of pregnancy (HR = 1.6 [1.3, 2.1]) than cows in the NE-VWP group. Median days to pregnancy were 93 [89, 103] d and 126 [116, 150] d, and mean days to pregnancy were 102.0 ± 2.0 d and 119.6 ± 2.3 d for E-VWP and NE-VWP, respectively.

As expected for TRM programs that provide different amounts of time for insemination at detected estrus for cows with or without estrus during the VWP [1,3], more cows received AIE for the group detected in estrus during the VWP. The latter were not only more likely to be cyclic, but also had two more weeks to be detected in estrus than cows with no estrus alerts during the VWP. More cyclic cows and more time for detection of estrus substantially reduced the need for TAI in cows with an AEA during the VWP as 83 % of these cows received AIE. On the other hand, for the NE-VWP group, fewer cyclic cows and a shorter period for detection of estrus resulted in a much smaller percentage of cows AIE than for cows with estrus during the VWP [−38 percentage points (p.p.)]. However, earlier submission to TAI for cows in the NE-VWP group helped maintain days to first service within 2.5 d of that of the E-VWP group. The combined effect of early first service for most cows that received AIE in the E-VWP and earlier enrollment into the TAI protocol for cows in the NE-VWP group contributed to reducing days to first service by almost a week for the TP-AIE treatment compared with the ALL-TAI treatment.

Collectively, data from this experiment conducted at a single commercial farm in Mexico demonstrated that a TRM program designed to optimize use of AIE with an automated system for detection of estrus and TAI based on estrus alerts during the VWP generated a similar pregnancy rate and proportion of cows pregnant by 150 d after calving compared with a program including all-TAI and an extended VWP. A combination

Table 4
Percentage of cows grouped based on the number of services received up to 150 DIM for the experimental treatments (ALL-TAI2 and TP-AIE3), estrus groups during the VWP (E-VWP5 and NE-VWP6), and parity group.

Variable	Outcome			
	Number of services up to 150 DIM, % (n/n)			
	1	2	3	≥4
Treatment[a]				
ALL-TAI[b]	43.0 (245/570)	33.7 (192/570)	19.8 (113/570)	3.5 (20/570)
TP-AIE[c]	33.4 (176/527)	34.0 (179/527)	22.4 (118/527)	10.2 (54/527)
E-group[d]				
E-VWP[e]	43.9 (292/665)	30.2 (201/665)	19.3 (128/665)	6.6 (44/665)
NE-VWP[f]	29.9 (129/432)	39.4 (170/432)	23.8 (103/432)	6.9 (30/432)
Parity				
Primiparous	46.7 (200/428)	32.9 (141/428)	15.9 (68/428)	4.4 (19/428)
Multiparous	33.0 (221/669)	34.4 (230/669)	24.4 (163/669)	8.2 (55/669)

[a] Treatment = treatment used for first service.
[b] ALL-TAI = cows received all TAI after a Double-Ovsynch protocol, TAI at 76 ± 3 DIM.
[c] TP-AIE = cows received AIE if detected in estrus for 30 ± 3 or 16 ± 3 d after a 49 d VWP. Cows not AIE with or without AEA during the VWP received TAI after P4-Ov at 90 ± 3 or 76 ± 3 DIM, respectively.
[d] E-group = cows grouped based on detection of estrus (yes vs. no) during the VWP (E-VWP vs. NE-VWP, respectively).
[e] E-VWP = cows with at least one estrus event based on AEA recorded during the VWP.
[f] NE-VWP = cows without an estrus event based on AEA recorded during the VWP.

of more cows with than without estrus alerts during the VWP, a short VWP, high estrus detection efficiency and P/AI for cows with estrus during the VWP, fewer days to first service, and earlier and more opportunities for reinsemination contributed to the success of the TRM program. Using a similar experimental design at a commercial dairy farm in the U.S., Rial et al. [3] recently demonstrated that a similar TRM program as that used in this experiment but using an ear-attached automated estrus detection system was as effective as an all-TAI program with an extended VWP. In contrast, Rial et al. [3] observed a greater HR for time to pregnancy for a TRM program than an all-TAI program with an extended VWP. Those results were due to relatively greater P/AI than in this experiment, especially for the NE-VWP (36.3 %) group. The favorable P/AI observed for the TRM program in Rial et al. [3] led to the same percentage of cows pregnant for the TRM and the ALL-TAI program. In contrast, in the current experiment more cows were pregnant (+9.2 p.p.) for the ALL-TAI than the TP-AIE treatment by the time that all cows received first service. These contrasting results between experiments highlight the need to maximize P/AI when using TRM programs that combine AIE and TAI. The latter must compensate not only for fewer P/AI at first service but also for the wider range of days to first service compared with programs that use all-TAI [5,13].

Several recent studies demonstrated that lactating dairy cows with automated estrus alerts during the VWP have superior reproductive performance than cows not detected in estrus [3,6]. Cows with estrus detected during the VWP were more likely to receive AIE [3,6], had more P/AI to first service [3], greater pregnancy rate [3,6], and more were pregnant by mid-to-late lactation [3,6]. In agreement, and in support of our secondary hypothesis, cows with at least one AEA during the VWP had better reproductive performance than cows without AEA regardless of the type of management. Cows in the E-VWP group were more likely to receive AIE for second service (26 p.p.), had greater P/AI after first (14 p.p.) and second service (10 p.p.), had greater pregnancy rate (60 % more likely to become pregnant per unit of time) and more cows were pregnant by 150 DIM (17 p.p.). These results provide additional evidence of the association between expression of estrus in early lactation and subsequent reproductive performance. The large differences observed between cows with and without estrus during the VWP suggested that dairy farms with automated estrus detection systems could use easily collected AEA data during the VWP as a decision-making tool for implementation of TRM programs for lactating cows.

Multiple biological factors could explain the contrast in reproductive performance between cows with and without an AEA during the VWP. In this experiment, cows without PVD, cows without lameness, and cows that maintained rather than lost or gained BCS were more likely to have an AEA during the VWP. Moreover, a greater proportion of cows with an AEA during the VWP were cyclic at every time point evaluated and cows with an AEA during the VWP had greater BCS before and at the time of first service. Earlier return to cyclicity postpartum and greater BCS before insemination are positively associated with fertility of lactating dairy cows [15–17] and could also explain part of the superior performance of cows with estrus during the VWP. We also observed that more primiparous than multiparous cows, more cows in the high tertile for DPR, and more cows without than with health disorders had an AEA during the VWP. Cows within groups with these favorable attributes had more P/AI to first service and more cows were pregnant by 150 DIM than their counterparts. Thus, these factors may not only be associated with a greater likelihood of having AEA during the VWP, but also contribute to the superior reproductive performance of the group with estrus during the VWP.

The multiple associations observed between cow features and physiological parameters with the likelihood of expression of estrus and with reproductive outcomes for cows with and without an AEA not only helps explain differences in reproductive performance but also suggests that these data collected during the VWP could be used in combination to form subgroups of cows for TRM [7,18,19]. Based on data from this experiment, parity group, the occurrence of health disorders through the VWP, and PTA for DPR should be included in algorithms combining multiple predictors for identifying cows with different reproductive potential. Records of PVD, lameness, and BCS could also be incorporated into algorithms if routinely collected by farms. Combining multiple sources of data for identifying groups of cows with different reproductive performance potential is supported by recent data from other studies. For example, Rial and Giordano [19] reported that combining data for expression of estrus during the VWP with other predictors, such as the occurrence of health disorders, genomic daughter pregnancy rate, and milk yield, generated subgroups of cows with larger differences in reproductive potential than if these predictors were used individually.

Data from this experiment also shed some light onto the association between observation of AEA during the VWP and cyclicity status of cows in the early postpartum period. Observing that 93.2 % of the cows with AEA during the VWP were cyclic demonstrated that a vast majority of cows with an AEA were likely in estrus and ovulated at least once during the VWP. Thus, a record of at least one AEA during a VWP of 50 d is a good indicator of return to ovarian cyclicity. A small proportion of cows can be expected to be incorrectly classified as cyclic based on AEA during the VWP because in our data 20 % of the non-cyclic cows based on the P4 sampling used in this experiment had an AEA. For these cows, which represent a small group of the total population (i.e., 4.1 %) we cannot rule out that management interventions that caused an increase in physical activity similar to episodes of increased activity observed during estrus were the reason for triggering the AEA. Moreover, it is possible that some cows with an AEA a few days before the second blood sampling time point did not yet have circulating concentrations of P4 above the cutoff to be considered cyclic despite having ovulated. On the other hand, 60.1 % of the cows with no AEA during the VWP and assigned to the NE-VWP were deemed cyclic based on circulating concentrations of P4. These observations are most likely explained by the biology of return to cyclicity and expression of estrus in the early postpartum period in dairy cattle and some of the criteria used to assign cows to the E-VWP and NE-VWP group. Specifically, lack of expression of estrus before the first postpartum ovulation (i.e., silent ovulation) in a substantial proportion of cows [20–22] could explain the lack of AEA in many cyclic cows. Other possible minor contributors to our observations were lack of estrus alerts in some cows that expressed estrus [23,24] and our choice to ignore certain estrus alerts (i.e., AEA before 15 DIM and on days around pen moves) to minimize the influence of false positive alerts on the TRM program. Ignoring certain alerts could have biased the classification of cows into the estrus or no estrus group during the VWP. Moreover, cows could have also been misdiagnosed as not in estrus by farm personnel that evaluated cows with an AEA through rectal palpation and visual observation of overt signs of estrus. Collectively, the present data suggested that the observation of at least one AEA during the VWP indicated with a high level of certainty that a cow returned to ovarian cyclicity but lack of an AEA did not imply lack of return to regular ovarian cyclicity. Despite this, the strong association between the occurrence of AEA during the VWP and reproductive performance supports the use of AEA during the VWP as a tool for creating groups for TRM. Future experiments should explore the potential value of combining cyclicity status based on plasma P4 concentrations with AEA during the VWP for creating groups for TRM.

5. Conclusions

A TRM program based on AEA during the VWP resulted in a high proportion of cows inseminated at detected estrus and reduced the proportion of cows that received TAI without detrimental effects on overall reproductive performance as compared with a first service program that increased first service P/AI through an all-TAI with a fertility protocol and an extended VWP. Use of AI at detected estrus for different lengths of time to cows with and without an AEA during the VWP prioritized use of synchronization of ovulation on cows more likely to benefit from TAI. Thus, the type of TRM program might be an alternative for dairy farms that strive to minimize use of hormonal interventions for TAI. This experiment also provided further evidence that records of AEA during the VWP are useful to identify cows with different reproductive potential.

CRediT authorship contribution statement

Ana Laura Laplacette: Writing – original draft, Project administration, Methodology, Investigation, Formal analysis, Data curation. **Clara Rial:** Methodology, Investigation, Formal analysis. **Gloria Stephanie Magaña Baños:** Project administration, Data curation. **José Alberto García Escalera:** Resources, Project administration. **Siddartha Torres:** Writing – review & editing, Resources, Project administration. **Allison Kerwin:** Writing – review & editing. **Julio Omar Giordano:** Writing – review & editing, Supervision, Methodology, Investigation, Funding acquisition, Conceptualization.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.theriogenology.2024.05.030.

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