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Interpretive Summary: Estrous Synchronization in Seasonal Calving Dairy Production Systems. Herlihy

Achieving a concentrated calving period in seasonal calving dairy production systems requires a high pregnancy rate within a short period following the planned start of mating. Reproductive performance following conventional estrous synchronization was compared with that after timed artificial insemination protocols. Timed artificial insemination protocols were associated with an increased likelihood of earlier conception after mating start date due to higher submission rates, shorter intervals from mating start date to conception and a higher proportion of animals successfully establishing pregnancy during the first 42 d of the breeding season.

ESTROUS SYNCHRONIZATION IN SEASONAL CALVING SYSTEMS

Evaluation of protocols to synchronize estrus and ovulation in seasonal calving pasture-based dairy production systems

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ABSTRACT

Lactating dairy cows (n=1,538) were enrolled in a randomized complete block design study to evaluate protocols to synchronize estrus and ovulation. Within each herd (n=8), cows were divided into three calving groups: EARLY, MID and LATE based on days in milk (DIM) at mating start date (MSD). EARLY calving cows (n=1,244) were ≥ 42 DIM at MSD, MID calving cows (n=179) were 21 to 41 DIM at MSD, and LATE calving cows (n=115) were 0 to 20 DIM at MSD. Cows in the EARLY, MID and LATE calving groups were synchronized to facilitate estrus or timed AI (TAI) at MSD (Planned Breeding 1; PB1), 21 d (PB2) and 42 d (PB3) after MSD, respectively. For each PB, cows in the relevant calving group were stratified by parity and calving date and randomly assigned to one of four experimental groups: 1) d -10 GnRH (10 µg i.m. Buserelin) and CIDR (Controlled Internal Drug Release) insert (1.38 g P4); d -3 $PGF_{2\alpha}$ (25 mg i.m. dinoprost); d -2 CIDR out and AI at observed estrus (CIDR_OBS); 2) same as CIDR_OBS, but GnRH 36 h after CIDR out and TAI 18 h later (CIDR_TAI); 3) same as CIDR_TAI, but no CIDR (OVSYNCH) or 4) untreated Controls (CONTROL). CIDR_OBS, CIDR_TAI and OVSYNCH had shorter mean intervals from calving to first service compared with CONTROL (69.2 d, 63.4 d, 63.7 d vs. 73.7 d, respectively). Both CIDR_OBS (predicted probability; PP of pregnancy = 0.59) and CIDR_TAI (PP of pregnancy = 0.54) had increased odds of conceiving to first service compared with OVSYNCH (PP of pregnancy = 0.45) (odds ratio; OR = 1.81 and OR = 1.46, respectively), and OVSYNCH had reduced likelihood of conceiving to first service (OR = 0.70) compared with CONTROL (PP of pregnancy = 0.53). Both CIDR_TAI (hazard ratio; HR (95% CI (confidence interval)) = 1.21 (1.04, 1.41)) and OVSYNCH (HR (95% CI) = 1.23 (1.05, 1.44)) were associated with an increased likelihood of earlier conception compared with CONTROL. A greater proportion of cows on the CIDR_TAI treatment successfully established pregnancy in the first 42 d of the breeding season compared with CONTROL (0.75 vs. 0.67 PP of 42-d pregnancy, respectively). Protocols to synchronize estrus and ovulation were effective at achieving earlier first service and conception in pasture-based seasonal calving dairy herds. However, animals that conceived following insemination at observed estrus had a reduced likelihood of embryo loss to first service compared with animals bred to TAI (PP of embryo loss to first service = 0.05 vs. 0.09; OR = 0.52).

Keywords: estrous synchronization, Ovsynch, dairy cow, seasonal calving

INTRODUCTION

Milk production in seasonal calving pasture-based systems (e.g., such as in Ireland) is dependent on the efficient conversion of grazed grass into milk (Dillon et al., 1995). Compact calving before turnout to pasture in spring is an essential component of pasture-based milk production systems to ensure maximum pasture utilization and hence profitability (Dillon et al., 1995). Achieving a highly concentrated period of calving in the spring requires a high pregnancy rate within a short period following the planned start of mating. Cows with North American genetics produced well in pasture-based systems of milk production (Horan et al., 2005a), but reproductive performance of such cows was well below optimum for seasonal calving systems (Horan et al., 2005b). Aggressive single-trait selection for increased milk production in Irish seasonal calving herds reduced profitability because the productivity gains were outweighed by increases in the costs associated with reproductive wastage (McCarthy et al., 2007).

Maximizing the proportion of cows that establish pregnancy within the first 42 d of the breeding season reduces the incidence of extended calving patterns (McDougall, 2006). Later calving cows with an extended postpartum anestrous interval can disrupt the seasonal calving pattern and result in extended calving patterns (Rhodes et al., 2003). Monitoring of submission rates in seasonal calving dairy herds provides a reliable indication of the efficiency and accuracy of estrous detection (Diskin and Sreenan, 2000). Low submission rates reduce the proportion of animals becoming pregnant within the pre-defined 42-d period, thus negatively impacting the profitability of seasonal calving systems. Reduced profitability arises from mean calving date (MCD) occurring later in the year than optimal, and consequently results in a less compact calving pattern. A study conducted in 74 Irish spring calving dairy herds (n=6,433 cows) reported that 81% of cows were detected in estrus and inseminated within the first 3 wk of the breeding season, 49% of cows conceive to first insemination, and 57% of cows are pregnant by 42 d after the start of the breeding season (Buckley et al., 2003).

Traditional estrous synchronization programs using GnRH, progesterone **(P4)** and $PGF_{2\alpha}$ successfully synchronized estrus and resulted in earlier conception in seasonal calving systems (Ryan et al., 1999; Ryan et al., 1995; Xu and Burton, 2000). Ovulation synchronization protocols using timed AI **(TAI)** ensure that a cow is submitted for AI without the requirement to observe for signs of estrus. The Ovsynch protocol includes an injection of GnRH 7 d before and 2 d after an injection of $PGF_{2\alpha}$, with TAI occurring between 16 to 18 h after the second GnRH injection (Pursley et al., 1995). Successful use of Ovsynch involves synchronizing: (i) the growth of a new follicular wave; (ii) induced luteal regression 7d later; and (iii) synchronization of ovulation 2 d later. Improved pregnancy outcomes following Ovsynch were reported when an intravaginal P4 insert was included during the treatment protocol for anovular cows (Chebel et al., 2010; McDougall, 2010; Stevenson et al., 2008) and cows with high P4 at the time of Controlled Internal Drug Release **(CIDR)** insertion that were more likely to undergo spontaneous corpus luteum regression before $PGF_{2\alpha}$ (Bartolome et al., 2009). The objective of this study was to examine the potential impact on calving pattern and MCD through aggressive whole herd intervention with protocols to synchronize estrus or ovulation. The results will be useful in benchmarking the effects of whole herd synchronization treatments in seasonal calving dairy production systems. This will be particularly useful for herds where MCD is currently later than desired.

MATERIALS AND METHODS

Farms and Animals

This study was conducted using 1,639 cows in 8 Irish commercial spring-calving dairy herds between April and June 2008. Within each farm, cows were managed as a single grazing herd and allocated fresh pasture twice daily as part of an intensively managed rotational grazing system with little or no concentrate supplementation. Breed compositions of the cows enrolled in the study comprised of Holstein-Friesian (n=1,173), Jersey × Holstein-Friesian crossbreds (n=284), Norwegian Red (n=16), Norwegian Red × Holstein-Friesian crossbreds (n=25) and "other" (n=141). The distribution of breeds on individual farms ranged from primarily Holstein-Friesian to primarily crossbreds. All experimental procedures involving animals were licensed in accordance with the Cruelty to Animals Act (Ireland 1897) and the European Community Directive 86/609/EC and were sanctioned by the University College Dublin Animal Research Ethics Committee. A clinical trials license was awarded by the Department of Agriculture, Fisheries and Food (Ireland) following approval by the Irish Medicines Board for the use of CIDR devices (1.38 g progesterone) that were undergoing registration approval at the time of the experiment.

Experimental Design and Treatments

All 1,639 lactating dairy cows were used in a completely randomised block experimental design to evaluate synchronization protocols. Within each herd, cows were divided into 3 groups: EARLY, MID and LATE calving based on DIM at the farm mating start date **(MSD)**. EARLY calving cows (n=1,301) were ≥ 42 DIM at MSD, MID calving cows (n=212) were 21 to 41 DIM at MSD, and LATE calving cows (n=126) were 0 to 20 DIM at MSD. Synchronization treatments commenced 10 d before MSD for the EARLY calving cows, facilitating estrus or timed artificial insemination **(TAI)** at MSD (Planned Breeding 1; PB1) as illustrated in Figure 1, upper panel. All EARLY calving cows were ≥ 42 DIM at AI (range in DIM of 42 to 105). Synchronization treatments commenced on d 11 and d 32 after MSD for the MID and LATE calving cows, respectively. The treatments facilitated estrus or TAI 21 d after MSD (PB2) and 42 d after MSD (PB3) for the MID and LATE calving cows, respectively. All MID and LATE calving cows were between 42 and 62 DIM at AI. Thus, the experimental treatments were imposed on all cows that had calved up to and including MSD.

Insert Figure 1 here

Synchronization Treatments and Artificial Insemination

Within each calving group, cows were stratified by parity and DIM and randomly assigned to one of the 4 treatments illustrated in Figure 1, lower panel. The **CIDR_OBS** treatment was an estrous synchronization protocol, whereas **CIDR_TAI** and **OVSYNCH** were ovulation synchronization protocols. The synchronization protocols were initiated at a random stage of the estrous cycle. Cows assigned to the **CONTROL** treatment (n=400) received no hormonal interventions. The i.m. GnRH agonist injections contained 10 µg buserelin (Receptal; Intervet Ireland, Dublin, Ireland). The CIDR device used contained 1.38 g of progesterone **(P4**; Pfizer Ireland, Dublin, Ireland). The i.m. $PGF_{2\alpha}$ contained 25 mg dinoprost tromethamine (Lutalyse; Pfizer Ireland, Dublin, Ireland). All hormonal treatments were administered by research staff from Teagasc Moorepark. Cows assigned to CONTROL and

CIDR_OBS were inseminated by the a.m./p.m. rule following detection of estrus with the aid of tail paint. All cows on the CIDR_TAI and OVSYNCH protocols received TAI 18 h after the second GnRH injection. The second GnRH injection was administered 60 h after $PGF_{2\alpha}$ as animals were only available at milking times; therefore, GnRH was administered after the evening milking as animals exited the milking parlor. All inseminations were performed by experienced technicians from commercial AI companies or by the herd owners and/or farm staff licensed by the Department of Agriculture, Fisheries and Food (Ireland) to carry out AI.

Transrectal Ultrasonography

The reproductive tracts of all cows were examined immediately before initiation of synchronization treatments by linear array ultrasonography using a 5.0-MHz transrectal transducer (Aloka SSD-500; Aloka Ltd., Tokyo, Japan). Cows were assigned an ultrasound reproductive tract score describing the volume and echogenicity of fluid contained within the uterus (Mee et. al., 2009). Cows that were classified as endometritic were not included in the study. To determine conception rates and embryo loss, all cows on synchronization treatments were scanned at 30 to 32 d and 56 to 58 d post AI. For CONTROL cows, the mean (and SD) days post AI at the corresponding scans were 41.2 (7.8) and 64.2 (6.2), respectively. Visualization of a fluid-filled uterine horn and the presence of a conceptus were used as positive indicators of pregnancy. For all cows in each herd, final pregnancy status was confirmed by palpation per rectum approximately six wk after the end of the breeding season.

Blood Collection and Progesterone Radioimmunoassay

Blood was collected in lithium heparin vacutainer tubes (Becton Dickinson, Plymouth, United Kingdom) by puncture of coccygeal vessels on the day of PB1 (EARLY cows), PB2 (MID cows), and PB3 (LATE cows) and again 11 d after PB1, PB2 and PB3. Blood samples were immediately placed in ice, and were later centrifuged at 2,000 × *g* for 15 minutes at 5 ^{o}C, the plasma was harvested and stored at -20 ^{o}C until later analysis. Concentrations of P4 in plasma were determined using a commercially available solid-phase radioimmunoassay (Coat-A-Count Progesterone, Diagnostic Products Corporation, Los Angles, CA). Sensitivity of the assay was 0.08 ng/mL; intra- and interassay coefficients of variation were 8.5 and 7.9%, respectively.

Reproductive Measurements

The following reproductive measurements were calculated and analyzed: 5-d submission relative to PB1, PB2, PB3 (binary); 21-d submission relative to PB1, PB2, PB3 (binary); overall 21-d submission (i.e., inseminated or not inseminated within the first 21 d of the breeding season irrespective of calving date; binary); calving to first service interval (**CSI**; interval in days from calving to first service; continuous); mating start date to conception (**MSDC**; interval in days from the mating start to conception determined by subsequent pregnancy detection; continuous); conception to first service (confirmed pregnant by ultrasonography at 30 to 32 d after first AI; binary); conception to second service (confirmed pregnant by ultrasonography at 30 to 32 d after second AI; binary); embryonic loss to first service (loss of a viable pregnancy between pregnancy diagnosis 1 (d 30 to 32 post-AI) and pregnancy diagnosis 2 (d 56 to 58 post-AI; binary); and 42-d pregnancy rate (successfully established pregnancy during the first 42 d of the breeding season; binary). When an individual cow received more than one insemination within a 4-d period, it was defined as one heat event and the later insemination date was used in the analysis.

Compliance to Protocol

Initially, 1,639 animals were enrolled in the study. However, 101 animals were subsequently removed from the dataset as they were not fully compliant with the designed protocol or were removed for other reasons described below. The breaches in protocol are illustrated in Figure 2, and included missed injections, mistimed CIDR removal, CIDR loss, and non-compliant inseminations. Animals considered unsuitable for breeding, determined by ultrasonography at the time of assignment to synchronization treatments were removed from the dataset. CONTROL animals administered injections were removed from the dataset. Animals with a missing value for conception rate to first service were removed from the dataset. After data edits, the final dataset included 1,538 cows used in protocols to synchronize estrus and ovulation. The numbers of animals reported per treatment were as follows: CIDR_OBS (n=398), CIDR_TAI (n=383), OVSYNCH (n=370), and CONTROL (n=387). The numbers of animals in the three calving groups that received synchronization treatments were as follows: EARLY (n=1,244), MID (n=179), and LATE (n=115).

Insert Figure 2 here

Synchronization Rate

Cows were categorized according to plasma P4 at d 0 (presumptive estrus) and d 11 after insemination (high [**H**] (≥1 ng/mL); low [**L**] (<1 ng/mL). Cows were grouped into P4 classes which resulted in four possible P4 class permutations for synchronized cows: HH, LL, HL, and LH. Only cows with L plasma P4 on d 0 and H plasma P4 on d 11 (i.e., LH) were considered synchronized. Of the 1,538 cows enrolled in the synchronization study, 1,506 (98%) cows were classified into one of the four P4 classes; at least one blood sample was missed for the remaining 32 (2%) cows. Progesterone concentrations in samples from CONTROL cows were used to determine cyclicity status and to determine the proportion of CONTROL cows that were cyclic / anestrous at each PB.

Statistical Analyses

Binary Traits. The effect of synchronization treatment and calving group (i.e., EARLY, MID, LATE) on the binary traits was determined using logistic regression with the GENMOD Procedure of SAS (SAS Inst. Inc., Cary, NC). A logit link function was used and a binomial distribution was assumed. The 8 binary traits were: 5-d submission rate relative to each PB, 21-d submission rate relative to each PB, overall 21-d submission rate, conception rate to first service, conception rate to second service, embryonic loss to first service, 42-d pregnancy rate, and synchronization rate. The logit of the probability of a positive outcome was modelled.

Model solutions were converted back to predicted probabilities by the formula

$$P = (1 + e^{-(\alpha+\beta x)})^{-1}$$

where α is the predicted intercept of the model, and β is the predicted regression coefficient(s) and x is the design matrix for the fixed effects in the model. The intercept represented the average farm and was representative of the parity and calving date structure in the data. Predicted probabilities may be interpreted as least squares means for the variable of interest estimated using linear models.

Odds ratios (**OR**) were calculated as the exponent of the model solutions. The odds ratio is an estimation of the relative odds of an event (i.e., likelihood of a positive outcome) occurring in the exposed group relative to a reference group or class. The CONTROL synchronization treatment and the EARLY calving group (≥ 42 DIM at MSD) were used as the reference groups for all variables with the exception of synchronization rate. For synchronization rate CONTROL animals were removed from the analysis and the OVSYNCH synchronization treatment and the EARLY calving group (≥ 42 DIM at MSD) were used as the reference groups. An odds ratio of 1 represents an equal likelihood of an event occurring to an animal in a particular group compared with a contemporary in the reference group. An odds ratio of >1 implies an increased likelihood of a positive outcome, whereas the opposite is true with an odds ratio of <1.

Explanatory independent variables considered for inclusion in all models included treatment (n=4), farm (n=8), parity of the cow (1, 2, 3, 4, ≥5), calving group (i.e., EARLY, MID, LATE), breed fraction of the cow as continuous variables (Holstein Friesian, Jersey, Norwegian Red and “other”), heterosis and recombination loss coefficients of the cow as continuous variables, an interaction term between synchronization treatment and calving group, and an interaction term between synchronization treatment and parity. Breed fraction, recorded in increments of 1/32, was fitted as a continuous variable to account for differences in the proportion of each breed (Holstein Friesian, Jersey, Norwegian Red and “other) in an animal; each breed was fitted as a separate covariate. Factors not associated (P > 0.05) with the dependent variables were removed by backward elimination. Preplanned contrasts were used to compare treatments to synchronize estrus and ovulation with the CONTROL treatment.

Non-Binary Traits. The effect of synchronization treatment and calving group on CSI was determined using a fixed effects linear model in the GLM procedure of SAS. (SAS Inst. Inc., Cary, NC). Explanatory independent variables considered for inclusion in the model were as before and included treatment (n=4), farm (n=8), parity of the cow (1, 2, 3, 4, ≥5), calving group (i.e., EARLY, MID, LATE), breed fraction of the cow as continuous variables (Holstein Friesian, Jersey, Norwegian Red and "other"), heterosis and recombination loss coefficients as continuous variables, an interaction term between synchronization treatment and calving group, and an interaction term between synchronization treatment and parity. Breed fraction, recorded in increments of 1/32, was fitted as a continuous variable to account for differences in the proportion of each breed (Holstein Friesian, Jersey, Norwegian Red and "other) in an animal; each breed was fitted as a separate effect in the model. Factors not associated ($P > 0.05$) with the dependent variables were removed by backward elimination.

Survival analysis was carried out using the Cox proportional hazard model in SAS (TPHREG procedure; SAS Inst. Inc., Cary, NC) to investigate the effect of synchronization treatment and calving group (i.e., EARLY, MID, LATE) on MSDC. In the analysis of MSDC, if a cow did not conceive to an insemination occurring during a 13-wk period from MSD, the data was right-censored at the maximum permissible value of 91 d (i.e., 13 wk). Explanatory independent variables considered for inclusion in the models were as before and included treatment (n=4), farm (n=8), parity of the cow (1, 2, 3, 4, ≥5), calving group (i.e., EARLY, MID, LATE), breed fraction of the cow as continuous variables (Holstein Friesian, Jersey, Norwegian Red and "other"), heterosis and recombination loss coefficients as continuous variables, an interaction term between synchronization treatment and calving group, and an interaction term between synchronization treatment and parity. Breed fraction, recorded in increments of 1/32, was fitted as a continuous variable, separate for each breed, to account for differences in the proportion of each breed (Holstein Friesian, Jersey, Norwegian Red and “other) in an animal. Factors not associated ($P > 0.05$) with the dependent variables were removed by backward elimination.

Survival was expressed as the relative hazard (Hazard Ratio; **HR**) of a cow conceiving at time (day) t, given that it had not conceived at day t -1 in the exposed group relative to the reference group. The CONTROL synchronization treatment and the EARLY calving group (≥ 42 DIM at MSD) were used as the reference groups. A hazard ratio of > 1 indicated that a unit increase in the value of the independent variable was associated with an increased likelihood of earlier occurrence of the event of interest. Predetermined contrasts were used to compare treatments to synchronize estrus and ovulation with the CONTROL treatment.

The interval from mating start date to conception (MSDC) was also evaluated by the LIFETEST procedure of SAS (SAS Inst. Inc., Cary, NC) using Kaplan-Meier analysis to investigate the effect of treatment on days from start of breeding to conception. The data are presented graphically as Survival Distribution Function by days after the planned start of mating for MSDC (Figure 3).

RESULTS

Reproduction and fertility performance

The explanatory independent variables included in the final model for all the fertility variables described above were treatment, farm, parity and calving group. The explanatory independent variables included in the final model for CSI and MSDC were treatment, farm, parity and calving group. The fixed effect of farm had a significant effect (*P* < 0.05) on synchronization rate and all fertility variables investigated with the exception of conception rate to second service, embryo loss to first service and 5-d submission rate relative to each PB. The fixed effect of parity had a significant effect (*P* < 0.05) on overall 21-d submission rate, 42-d pregnancy rate, CSI, MSDC and synchronization rate and had no effect on the remaining fertility variables. With the exception of synchronization rate and CSI, a significant parity effect for the variables listed was reflected by better performance in lower parity animals compared with older animals. Proportion of Jersey was associated (*P* = 0.03) with 5-d submission rate relative to each PB (regression coefficient of 0.0324; SE = 0.0153) while proportion Holstein Friesian was associated (*P* = 0.02) with 21-d submission relative to each PB (regression coefficient of -0.0270; SE = 0.0114). Also, proportion Holstein Friesian was associated (*P* = 0.02) with conception rate to first service (regression coefficient of the logit of the probability of conception of -0.0176; SE = 0.0076). The coefficient of recombination loss and proportion Jersey was associated (*P* = 0.04 and *P* = 0.01, respectively) with 42-d pregnancy rate (regression coefficient of the logit of the probability of pregnant of 1.0322; SE = 0.5161 and 0.0302; SE = 0.0119), respectively while the coefficient of heterosis and Jersey proportion were associated (*P* = 0.04 and *P* = 0.01, respectively) with the interval from MSDC (regression coefficient 0.22067; SE = 0.10921 and 0.01295; SE = 0.00522), respectively.

The effect of synchronization treatment on 5-d and 21-d submission rate relative to each PB for CIDR_OBS and CONTROL is summarized in Table 1. The intercept of the multiple regression model for 5-d and 21-d submission rate relative to each PB was -0.65 (SE = 0.3) and 2.33 (SE = 0.4), respectively. Both TAI protocols resulted in 5-d and 21-d submission rates relative to each PB of 1.00. Synchronization treatment (*P* < 0.001), calving group (*P* = 0.009) and their interaction (*P* = 0.056) had significant effects on 5-d submission rate relative to each PB. CIDR_OBS had increased odds of being submitted for insemination in the first 5-d relative to each PB compared with CONTROL (*P* < 0.001). The significant interaction observed was due to the lower 5-d submission rate for CONTROL animals in the MID calving group relative to CONTROL animals in the EARLY and LATE calving groups, whereas the 5-d submission rate was similar for all calving groups on the CIDR_OBS treatment. The 5-d submission rate relative to each PB for CONTROL animals in the MID and LATE calving groups represents the proportion of CONTROL animals inseminated during the 5-d period following PB2 and PB3. However, CONTROL animals in the MID and LATE calving groups were eligible for AI from the time CIDR_OBS, CIDR_TAI and OVSYNCH were assigned to synchronization treatments on d 11 and d 32, respectively. If the CONTROL animals inseminated in the 10-d period that synchronization treatments were imposed were reported, an additional 23 (MID) and 9 (LATE) CONTROL cows would have been included, increasing 5-d submission rate for CONTROL cows in the MID and LATE calving groups to 0.63 and 0.75, respectively.

Synchronization treatment (*P* = 0.04), calving group (*P* < 0.001) and their interaction (*P* < 0.001) had significant effects on 21-d submission rate relative to each PB. CIDR_OBS had increased odds of being submitted for insemination in the first 21-d relative to each PB compared with CONTROL (*P* = 0.04). The observed significant interaction was due to the lower 21-d submission rate for CONTROL animals in the MID and LATE calving groups compared to the EARLY calving group, whereas the 21-d submission rate was similar for all calving groups on the CIDR_OBS treatment. The 21-d submission rate relative to each PB for CONTROL animals in the MID and LATE calving groups represents the proportion of CONTROL animals inseminated during the 21-d period following PB2 and PB3. However, CONTROL animals in the MID and LATE calving groups were eligible for AI from the time CIDR_OBS, CIDR_TAI and OVSYNCH were assigned to synchronization treatments on d 11 and d 32, respectively. If the CONTROL animals inseminated in the 10-d period that synchronization treatments were imposed were reported, the inclusion of an additional 23 (MID) and 9 (LATE) CONTROL cows would have increased 21-d submission rate for CONTROL cows in the MID and LATE calving groups to 0.77 and 0.97, respectively.

The effect of synchronization treatment on overall 21-d submission rate is summarized in Table 1. The intercept of the multiple regression model for overall 21-d submission rate was 1.50 (SE = 0.29). Due to a confounding effect between calving group and overall 21-d submission rate, calving group was removed from the statistical model for this variable. Synchronization treatment had a significant effect on overall 21-d submission rate ($P < 0.001$). Both CIDR_TAI and OVSYNCH had increased odds of being submitted for insemination in the first 21-d of the breeding season compared with CONTROL (both $P < 0.001$). CIDR_OBS had reduced likelihood of being submitted for insemination in the first 21-d of the breeding season compared with CIDR_TAI and OVSYNCH (OR = 0.26, $P < 0.001$; and OR = 0.25, $P < 0.001$, respectively).

The effect of synchronization treatment on conception rate to first service is summarized in Table 2. The intercept of the multiple regression model for conception rate to first service was 0.15 (SE = 0.2). Synchronization treatment had a significant effect on conception rate to first service ($P = 0.0009$), but calving group and the interaction between synchronization treatment and calving group were not significant

(*P* = 0.8 and *P* = 0.3, respectively). Both CIDR_OBS and CIDR TAI had increased odds of conceiving to first service compared with OVSYNCH (OR = 1.81, *P* < 0.001; and OR = 1.46, *P* = 0.01, respectively), and OVSYNCH had reduced likelihood of conceiving to first service compared with CONTROL (OR = 0.70, *P* = 0.02). Animals inseminated based on observed estrus had an increased likelihood of conceiving to first service compared with animals bred to TAI (OR = 1.33, *P* = 0.007). There was no effect of synchronization treatment (*P* = 0.8), calving group (*P* = 0.8) or their interaction (*P* = 0.3) on conception rate to second service, and none of the synchronization treatments had odds ratios that differed from the CONTROL treatment. The intercept of the multiple regression model for conception rate to second service was 0.18 (SE = 0.3). Mean conception rate at second AI across all treatments was 0.56.

The effect of synchronization treatment on embryo loss to first service is summarized in Table 3. The intercept of the multiple regression model for embryo loss to first service was -3.36 (SE = 0.6). Synchronization treatment had a significant effect on embryo loss to first service (*P* = 0.05), but calving group and the interaction between synchronization treatment and calving group were not significant (*P* = 0.6 and *P* = 0.9, respectively). OVSYNCH had increased odds of embryo loss to first service (*P* = 0.0097) compared with CONTROL. Both CIDR_OBS and CIDR_TAI tended to have an increased odds of embryo loss to first service compared with CONTROL (*P* = 0.10 and *P* = 0.07, respectively). CONTROL had reduced likelihood of embryo loss to first service compared with animals bred to either TAI protocols (OR = 0.35, *P* = 0.02) or CIDR-based protocols (OR = 0.44, *P* = 0.06). Animals that conceived following insemination at observed estrus had a reduced likelihood of embryo loss to first service compared with animals bred to TAI (PP of embryo loss to first service = 0.05 vs. 0.09; OR = 0.52, $P = 0.03$).

The effect of synchronization treatment on 42-d pregnancy rate is summarized in Table 4. The intercept of the multiple regression model for 42-d pregnancy rate was 1.01 (SE = 0.2). There was no overall effect of synchronization treatment on 42-d pregnancy rate ($P = 0.11$); however, the CIDR_TAI treatment resulted in greater 42-d pregnancy rate compared with CONTROL. None of the other treatments differed from each other. Calving group had a significant effect on 42-d pregnancy rate ($P < 0.001$), and the interaction between synchronization treatment and calving group tended towards significance ($P = 0.08$). This was due to the tendency for greater 42-d pregnancy rates in the synchronized animals in the MID and LATE groups compared with CONTROL.

Synchronization treatment and calving group had a significant effect on CSI (both $P < 0.001$), but the interaction between synchronization treatment and calving group was not significant ($P = 0.3$). The intercept of the fixed effects linear model for CSI was 77.88 d (SE = 1.4 d). Least squares means (± SE) for CSI were 69.2 d (0.7), 63.4 d (0.7), 63.7 d (0.7) and 73.7 d (0.7) for CIDR_OBS, CIDR_TAI, OVSYNCH and CONTROL, respectively. All synchronization treatments had shorter ($P < 0.001$) intervals from calving to first service compared with CONTROL. CIDR_TAI and OSYNCH had shorter CSI compared with CIDR_OBS ($P < 0.001$), and CIDR_TAI and OVSYNCH did not differ ($P = 0.8$).

Synchronization treatment ($P = 0.03$) and calving group ($P < 0.001$) affected the interval from MSDC, but the interaction term was not significant ($P = 0.8$). Both CIDR_TAI (HR (95% CI) = 1.21 (1.04, 1.41), $P = 0.02$) and OVSYNCH (HR (95% CI) = 1.23 (1.05, 1.44), $P = 0.0089$) were associated with an increased likelihood of earlier conception compared with CONTROL (Figure 3). A tendency for increased likelihood of earlier conception was observed for CIDR_OBS compared with CONTROL (HR (95% CI) = 1.15 (0.99, 1.34), $P = 0.06$). CONTROL had reduced likelihood of earlier conception compared with animals bred to TAI (HR = 0.82, $P = 0.003$). Animals inseminated based on observed estrus had a reduced likelihood of earlier conception compared with animals bred to TAI (HR = 0.88, $P = 0.02$). CONTROL had reduced likelihood of earlier conception compared with animals assigned to the CIDR based protocols (HR = 0.85, $P = 0.01$). The median MSDC for CIDR_OBS, CIDR_TAI, OVSYNCH and CONTROL was 33.2 d, 30.9 d, 32.1 d and 37.1 d, respectively.

Insert Figure 3 here

The effect of synchronization treatment on synchronization rate is summarized in Table 5. The intercept of the multiple regression model was 2.03 (SE = 0.4). Synchronization treatment had a significant effect on synchronization rate ($P < 0.001$), but calving group and the interaction between synchronization treatment and calving group were not significant ($P = 0.19$ and $P = 0.13$, respectively). The proportion of animals on synchronization treatments in the P4 categories were as follows: LH (n=1,012; 89.4%), LL (n=100; 8.83%), HL (n=8; 0.71%) and HH (n=12; 1.06%). CIDR_TAI had increased likelihood of being synchronized compared with CIDR_OBS (OR = 3.79, $P < 0.001$) and OVSYNCH (OR = 4.50, $P < 0.001$), but there was no difference between CIDR_OBS and OVSYNCH ($P = 0.4$). The proportion of CONTROL animals in the P4 categories were as follows: LH (n=115; 30.8%), LL (n=54; 14.4%), HL (n=65; 17.4%) and HH (n=140; 37.4%). Therefore, 85.6% of the CONTROL cows were considered to be cycling normally during the period of synchronization treatments.

DISCUSSION

The present study compared the reproductive performance of seasonal calving lactating dairy cows following treatment with protocols to synchronize estrus or ovulation with that of non-synchronized cows. This study provided a valuable opportunity to investigate the potential of aggressive synchronization as a tool to alter the calving pattern of dairy cows in seasonal calving systems. Experimental treatments were imposed on all cows that had calved up to and including the MSD, thus maximizing the proportion of the herd bred to AI during the first 42 d of the breeding season, a parameter of particular importance in seasonal calving herds. Use of TAI protocols resulted in shorter intervals from calving to first service and from mating start date to conception. Progesterone supplementation as part of a TAI protocol resulted in a higher proportion of these animals successfully establishing pregnancy during the first 42 d of the breeding season.

Achieving high submission rates within the first 21 d of the breeding season is a prerequisite for a compact calving pattern the following spring (Diskin and Sreenan, 2000). The overall 21-d submission rate for animals in the present study was in line with targets set down for seasonal calving systems (McDougall, 2006), and similar to submission rates recently achieved on Irish dairy farms (Buckley et al., 2003). The 21-d submission rate for CONTROL cows in the EARLY calving group, which represented CONTROL animals calved the longest period of time, was in line with targets for seasonal calving systems (McDougall, 2006). However, CONTROL animals in the MID and LATE calving groups had lower submission rates, presumably reflecting closer proximity to calving in these groups compared with the EARLY calving group. These results highlight the considerable challenge associated with later calving cows in seasonal calving systems (Grosshans et al., 1997). The 5-d submission rate relative to each PB for CIDR_OBS averaged 0.81, and indicated that an acceptable proportion of animals displayed estrus and were submitted for insemination within the first 5-d relative to each PB. In the present study, the CIDR device was inserted for 8 d and removed 1 d after the $PGF_{2\alpha}$ injection based on previous reports of improved precision in the onset of estrus when CIDR inserts were removed after an 8 d treatment period (Xu and Burton, 2000). Using a similar protocol, Ryan et al. (1995) and Ryan et al. (1999) reported that 88.5% and 87.5% of animals, respectively, were detected in estrus and submitted for insemination by d 4 after the start of the breeding period.

The challenge of low submission rates can be overcome by incorporating TAI protocols into reproductive management programs (Lucy et al., 2004). Ovsynch has been successfully used for synchronizing follicular wave development, luteolysis, and ovulation in lactating dairy cows (Pursley et al., 1997; Pursley et al., 1995). The use of TAI protocols in the current study ensured that all animals assigned to TAI protocols in the EARLY, MID and LATE calving groups were submitted for insemination on PB1, PB2 and PB3, respectively. While a significant improvement in submission rate was achieved with the use of CIDR_OBS compared with CONTROL, the values for 5-d (0.81 vs. 0.33) and 21-d (0.89 vs. 0.84) submission rates relative to each PB were considerably lower than the pre-determined value of 1 for animals assigned to TAI protocols. The positive impact of TAI protocols on submission rate was particularly apparent when evaluating 5-d submission rate for CIDR_OBS in the MID (0.77) and LATE (0.74) calving groups. The use of TAI protocols resulted in more cows submitted for insemination earlier in the breeding season compared with CIDR_OBS and CONTROL. An increase in submission rates with TAI protocols was observed for all calving groups, but the impact was greatest in the later calving cows. A major limitation of the CIDR_OBS protocol was that the submission rate achieved was dependent on estrous behavior and estrus detection efficiency.

The conception rate to first service of cows assigned to OVSYNCH was lower when compared with all other treatments. In agreement with previous studies, P4 supplementation during the treatment protocol was associated with more favorable pregnancy outcomes compared with OVSYNCH, whether animals receiving supplemental P4 were inseminated based on observed estrus or TAI (Chebel et al., 2010; McDougall, 2010; Melendez et al., 2006). The highest conception rate to first service was obtained with the CIDR_OBS protocol. Conception rate to first service for CIDR_OBS in the current study was similar to that in the first of two trials reported by Xu and Burton (2000) (56.5%) where animals were treated with GnRH and an intravaginal P4 device followed 7 d later by $PGF_{2\alpha}$, and removal of the P4 device 1 d after $PGF_{2\alpha}$. However in the second trial Xu and Burton (2000) reported an improvement in conception rates (64.6%) when the duration of P4 treatment was reduced from 8 d to 7 d and CIDR removal occurred concurrent with $PGF_{2\alpha}$ injection. Xu and Burton (2000) concluded that the extra day of P4 treatment after $PGF_{2\alpha}$ injection in the first trial may have allowed some dominant follicles to be maintained for a longer period, resulting in the ovulation of aged oocytes with reduced developmental competence. Using a comparable protocol to CIDR_OBS (Ryan et al., 1995) reported similar pregnancy rates (57.9%); however, a protocol that did not include GnRH at the time of CIDR insertion was associated with an 11- to 14-percentage unit reduction in pregnancy rates (46.6%).

Addition of P4 to Ovsynch (CIDR_TAI) resulted in 0.09 greater first service conception rate when compared with OVSYNCH. In agreement with the results from the current study and the majority of studies not using presynchronization, the first of two experiments completed by El-Zarkouny et al. (2004) reported higher pregnancy rates at 29 d post-AI (59.3 vs. 36.3%) for animals supplemented with P4 during Ovsynch compared with animals treated with the standard Ovsynch protocol. However, in a second experiment, when presynchronization was used, El-Zarkouny et al. (2004) reported that P4 supplementation appeared to offer no improvement in pregnancies per AI over Ovsynch alone. McDougall (2010) reported that addition of P4 to Ovsynch for anestrous cows tended to increase 21 day pregnancy rate compared with anestrous cows treated with Ovsynch (57.5 vs. 48.4%). In the same study, addition of P4 to Ovsynch resulted in more cows with normal subsequent luteal-phase lengths. An 8.5-percentage unit improvement in pregnancy rate was reported by Melendez et al. (2006) for animals not previously detected in estrus following presynchronization, that were supplemented with P4 during Ovsynch compared with animals treated with Ovsynch alone (31.2 vs. 22.7%). Following a $PGF_{2\alpha}$ based presynchronization protocol, Stevenson et al. (2008) compared pregnancies per AI in cows without a corpus luteum at the first GnRH injection of Ovsynch, receiving or not receiving 7 d P4 supplementation via a CIDR insert with that of cows with a corpus luteum present. It was reported that treatment with a CIDR in cows without a corpus luteum increased pregnancies per AI at both 33 and 61 d after TAI, but did not differ from that of cows that had a corpus luteum present at the time of the first GnRH injection of Ovsynch.

In the current study, across ovular and anovular cows at protocol initiation, conception rate to first service using the Ovsynch TAI protocol was similar to that reported by Cordoba and Fricke (2001) for ovular cows managed in grazing based dairies in Wisconsin. In other studies, conception rates have ranged between 31.3 to 45.0% following the Ovsynch protocol initiated at random stages of the estrous cycle (McDougall, 2010; Peters and Pursley, 2002, 2003; Pursley et al., 1997; Pursley et al., 1998). Lower conception rates following Ovsynch have been reported for anovular cows, possibly due to a higher incidence of premature luteal regression (Gumen et al., 2003). Vasconcelos et al. (1999) reported that initiation of Ovsynch on different days of the estrous cycle affected pregnancy outcome arising from variation in ovulatory responses to the first and second GnRH and maximal size of the pre-ovulatory follicle. In the present study, synchronization protocols were initiated at random stages of the estrous cycle with no presynchronization before initiation of synchronization protocols.

The embryo loss rate to first service in the current study was generally low; values were similar for all treatments with the exception of OVSYNCH, which had 0.07 greater embryo loss compared with CONTROL. The embryo loss rate for CONTROL animals in the current study was lower than the embryonic loss rate of 7.2% between d 28 and 84 of gestation previously reported in Irish pasture-based herds (Silke et al., 2002) and much lower than embryonic loss rates reported by Gumen et al. (2003) for ovular cows maintained in high input TMR system that were inseminated based on observed estrus or TAI (11 vs. 14%, respectively). The CONTROL animals in the present study were inseminated based on observed estrus. For logistical reasons, it was not possible to carry out the pregnancy diagnosis for CONTROL cows with the same level of precision as synchrony cows for days post-AI at pregnancy diagnosis. Consequently, both the conception to AI and the embryo loss rate for CONTROL animals in the present study may have been slightly underestimated relative to the synchrony treatments. In agreement with McDougall (2010), embryo loss rate did not differ between OVSYNCH and CIDR_TAI. In a recent review Santos et al. (2004) concluded that the majority of studies that implement TAI protocols have reported no difference in embryonic loss rates when timed AI has been implemented properly. In the same review, the authors suggested that synchronization protocols that induce estrus with the dominant follicle growing under a low P4 environment may increase early and late embryo loss, leading to reduced conception rates.

In the present study, only 0.42 and 0.27 of MID and LATE calving CONTROL cows successfully established pregnancy during the first 42 d of the breeding season. Conception rate to first service for CONTROL animals was consistent across all calving groups. The reduced submission rates for CONTROL cows in the MID and LATE calving groups therefore contributed to a significant reduction in the proportion of CONTROL cows successfully establishing pregnancy during the first 42 d of the breeding season. In contrast, a similar conception rate to first service coupled with a 100% submission rate for all calving groups resulted in CIDR_TAI having the highest 42-d pregnancy rate, which is in agreement with the findings of McDougall (2010). A shorter interval from MSD to conception was observed for animals assigned to TAI protocols when compared with CONTROL, similar to the findings of McDougall (2010). In the present study, it is important to note that the submission rate figures for the cows on the CONTROL treatment met targets laid down for seasonal calving systems. Where herds do not routinely meet these targets, the potential impact of aggressive whole herd synchronization incorporating TAI is increased proportionate to the increase in submission rate achieved.

CONCLUSIONS

The present study clearly shows that estrus/ovulation can be successfully synchronized with progesterone, GnRH and $PGF_{2\alpha}$ in seasonal calving dairy cows. Reliance on behavioral estrus/estrus detection limits the submission rates that can be achieved with conventional synchronization protocols. In contrast, TAI protocols ensure that submission rates are maximised, while maintaining acceptable conception rates. Importantly, TAI protocols facilitated earlier first service and earlier conception, increasing the proportion of cows establishing pregnancy during the critical first 42 d of the breeding season. Supplementation with progesterone during Ovsynch (i.e., CIDR_TAI) increased conception rates. In conclusion, ovulation synchronization protocols are an effective tool in the reproductive management of lactating dairy cows in seasonal calving, pasture-based milk production systems.

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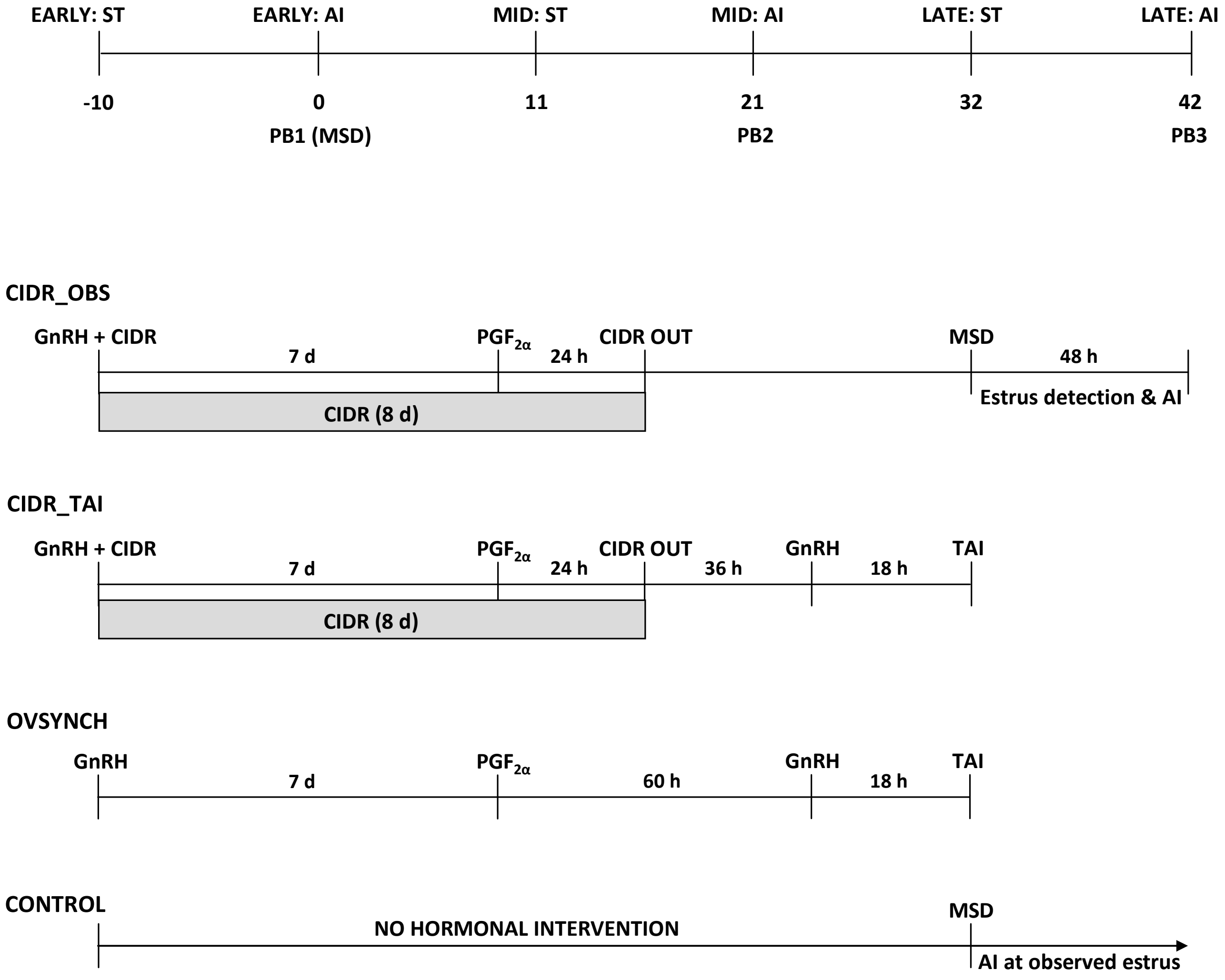

Figure 1: Schematic diagram of experimental design used to evaluate synchronization treatments (ST) (upper panel) and treatment protocols to synchronize estrus and ovulation (lower panel)**.** EARLY calving cows were ≥ 42 DIM at MSD, MID calving cows were 21 to 41 DIM at MSD, and LATE calving cows were 0 to 20 DIM at MSD. PB refers to Planned Breeding 1 (MSD), 2 and 3. For each seasonal calving farm in the study (n=8) breeding started on a fixed calendar date, referred to as the Mating Start Date (MSD). In this study, PB1 coincided with the MSD for each farm. PB2 occurred 21 d after PB1, and PB3 occurred 42 d after PB1 or 21 d after PB2. Treatment protocols for synchronization were initiated at a random stage of the

estrous cycle and applied to lactating dairy cows before first service. **CIDR_OBS** (10 µg GnRH and CIDR insert d 0, 25 mg $PGF_{2\alpha}$ d 7, CIDR removed d 8, animals were inseminated by the a.m./p.m. rule following detection of estrus on d 10, 11 and 12). **CIDR_TAI** (10 µg GnRH and CIDR insert d 0, 25 mg $PGF_{2\alpha}$ d 7, CIDR removed d 8, 10 µg GnRH 60 h after $PGF_{2\alpha}$ or 36 h after CIDR removal, animals received TAI 18 h after the final GnRH). **OVSYNCH** (10 µg GnRH d 0, 25 mg $PGF_{2\alpha}$ d 7, 10 µg GnRH 60 h after $PGF_{2\alpha}$, animals received TAI 18 h after the final GnRH).

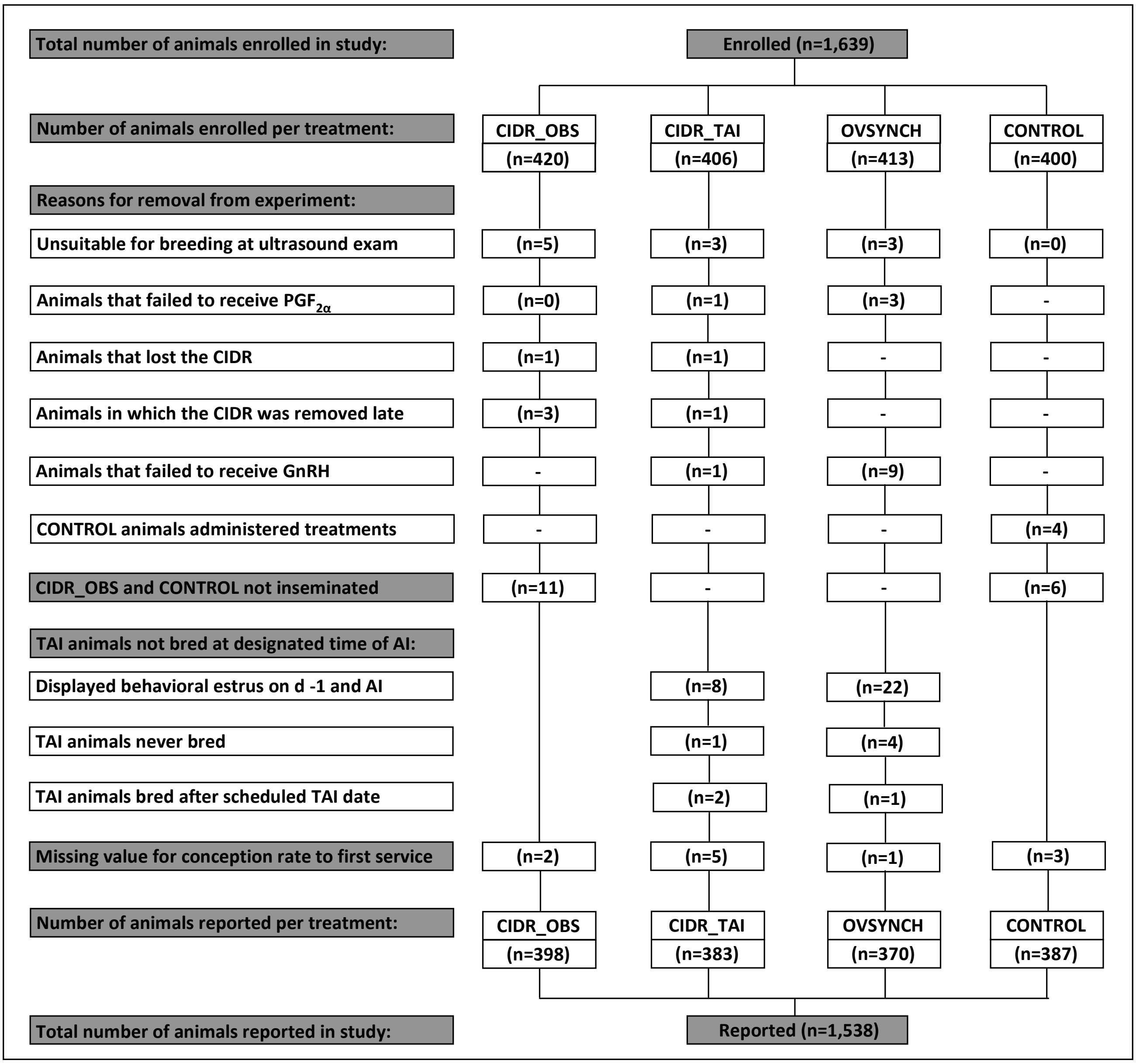

Figure 2. Flowchart showing assignment of animals to treatment protocols to synchronize estrus and ovulation. After data edits the final dataset included 1,538 cows used in protocols to synchronize estrus and ovulation. The number of animals in the three calving groups that received synchronization treatments were as follows: EARLY calving (GROUP 1) (n=1,244), MID calving (GROUP 2) (n=179), and LATE calving (GROUP 3) (n=115).

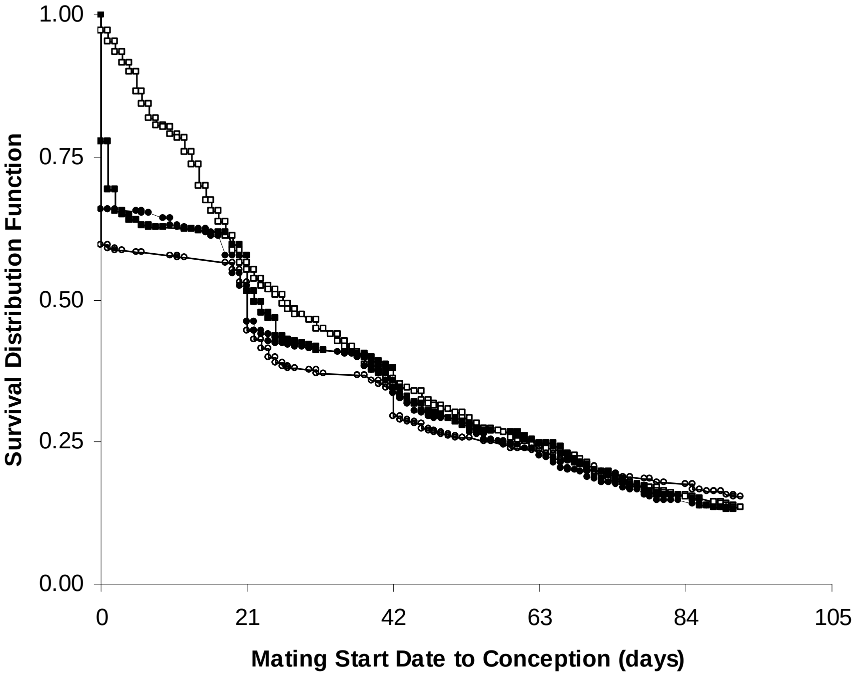

Figure 3. Survival distribution function for the interval in days from mating start date to conception (MSDC) for CIDR_OBS (■), CIDR_TAI (○), OVSYNCH (●) and CONTROL (□).

Table 1. Effect of synchronization treatment and calving group on 5-d submission rate (SR) relative to each planned breeding (PB), 21-d SR relative to each PB and overall 21-d SR relative to MSD[‡]

		Predicted probability[2] (Standard error)			
Synchronization Treatment	Odds Ratio 95% CI[1]	ALL COWS	EARLY	MID	LATE
5-d submission rate for each planned breeding[†]					
CIDR_OBS	8.73 (6.22,12.25)	0.81^{a} (0.02)	0.83^{a} (0.02)	0.77^{a} (0.06)	0.74^{a} (0.08)
CONTROL	1.00 -	0.33^{b} (0.02)	0.37^{bx} (0.03)	0.11^{by} (0.05)	0.41^{bx} (0.09)
21-d submission rate for each planned breeding[†]					
CIDR_OBS	1.55 (1.02,2.34)	0.89^{a} (0.02)	0.90 (0.02)	0.89^{a} (0.05)	0.83 (0.06)
CONTROL	1.00 -	0.84^{b} (0.02)	0.93^{x} (0.01)	0.28^{by} (0.07)	0.67^{z} (0.09)
Overall 21-d submission rate relative to mating start date					
CIDR_OBS	0.82 (0.58,1.16)	0.78^{a} (0.02)	-	-	-
CIDR_TAI	3.15 (1.98,5.00)	0.93^{b} (0.01)	-	-	-
OVSYNCH	3.32 (2.06,5.34)	0.94^{b} (0.01)	-	-	-
CONTROL	1.00 -	0.82^{a} (0.02)	-	-	-

[1] CI = Confidence Interval.

[2] Predicted Probabilities are based on a cow from the average farm and are representative of the parity and calving date structure in the data.

[†] For CIDR_TAI and OVSYNCH 5-d and 21-d submission rate relative to each PB was 1.00, and hence these animals were removed from the analysis of 5-d and 21-d submission rate relative to each PB.

[a,b] Predicted probabilities within a column with different superscripts differ ($P < 0.05$).

^{x,y,z} Predicted probabilities within a row with different superscripts differ ($P < 0.05$).

[‡] Interaction between synchronization treatment and calving group: 5-d SR for each PB ($P = 0.055$); 21-d SR for each PB ($P < 0.001$).

Table 2. Effect of synchronization treatment and calving group on conception rate to first service[‡]

Synchronization Treatment	Odds Ratio 95% CI[1]	Predicted probability[2] (Standard error)			
		ALL COWS	EARLY	MID	LATE
CIDR_OBS	1.28 (0.96,1.70)	0.59[a] (0.02)	0.58 (0.03)	0.72 (0.07)	0.53 (0.09)
CIDR_TAI	1.03 (0.77,1.37)	0.54[a] (0.03)	0.54 (0.03)	0.51 (0.08)	0.58 (0.09)
OVSYNCH	0.70 (0.53,0.94)	0.45[b] (0.03)	0.47 (0.03)	0.35 (0.07)	0.33 (0.09)
CONTROL	1.00 -	0.53[a] (0.03)	0.55 (0.03)	0.47 (0.07)	0.54 (0.10)

[1] CI = Confidence Interval.

[2] Predicted Probabilities are based on a cow from the average farm and are representative of the parity and calving date structure in the data.

[a,b] Predicted probabilities within a column with different superscripts differ ($P < 0.05$).

[‡] Interaction between synchronization treatment and calving group ($P = 0.2$).

Table 3. Effect of synchronization treatment and calving group on embryo loss to first service[‡]

Synchronization Treatment	Odds Ratio 95% CI[1]	Predicted probability[2] (Standard error)			
		ALL COWS	EARLY	MID	LATE
CIDR_OBS	2.16 (0.86,5.43)	0.06^{ab} (0.02)	0.06 (0.02)	0.10 (0.05)	0.05 (0.05)
CIDR_TAI	2.38 (0.94,6.01)	0.07^{ab} (0.02)	0.08 (0.02)	0 [†]	0.06 (0.06)
OVSYNCH	3.35 (1.34,8.35)	0.10^{a} (0.02)	0.12 (0.03)	0 [†]	0 [†]
CONTROL	1.00 -	0.03^{b} (0.01)	0.03 (0.01)	0.05 (0.05)	0.06 (0.06)

[1] CI = Confidence Interval.

[2] Predicted Probabilities are based on a cow from the average farm and are representative of the parity and calving date structure in the data.

[†] None of the animals on this synchronization treatment and in this group underwent embryo loss to first service and hence these animals were subsequently removed from analysis investigating synchronization treatment and group interaction effects.

[a,b] Predicted probabilities within a column with different superscripts differ ($P < 0.05$).

[‡] Interaction between synchronization treatment and calving group ($P = 0.9$).

Table 4. Effect of synchronization treatment and calving group on 42-d pregnancy rate[‡]

Synchronization Treatment	Odds Ratio 95% CI[1]	Predicted probability[2] (Standard error)			
		ALL COWS	EARLY	MID	LATE
CIDR_OBS	1.23 (0.89,1.70)	0.71^{bc} (0.02)	0.76^{x} (0.02)	0.69^{ax} (0.07)	0.32^{ay} (0.08)
CIDR_TAI	1.52 (1.09,2.12)	0.75^{ac} (0.02)	0.78^{x} (0.02)	0.64^{aby} (0.07)	0.58^{by} (0.09)
OVSYNCH	1.25 (0.90,1.74)	0.71^{bc} (0.02)	0.79^{x} (0.02)	0.48^{bcy} (0.07)	0.33^{aby} (0.09)
CONTROL	1.00 -	0.67^{b} (0.02)	0.75^{x} (0.02)	0.42^{cy} (0.07)	0.27^{ay} (0.09)

[1] CI = Confidence Interval.

[2] Predicted Probabilities are based on a cow from the average farm and are representative of the parity and calving date structure in the data.

[a,b,c] Predicted probabilities within a column with different superscripts differ ($P < 0.05$).

[x,y] Predicted probabilities within a row with different superscripts differ ($P < 0.05$).

[‡] Interaction between synchronization treatment and calving group ($P = 0.08$).

Table 5. Effect of synchronization treatment and calving group on synchronization rate[‡]

		Predicted probability[2] (Standard error)			
Synchronization Treatment	Odds Ratio 95% CI[1]	ALL COWS	EARLY	MID	LATE
CIDR_OBS	1.19 (0.78, 1.82)	0.90[a] (0.02)	0.91 (0.02)	0.87 (0.05)	0.84 (0.06)
CIDR_TAI	4.50 (2.47, 8.20)	0.97[b] (0.01)	0.97 (0.01)	0.97 (0.03)	1.00 (0)
OVSYNCH	1.00 -	0.88[a] (0.02)	0.89 (0.02)	0.93 (0.04)	0.72 (0.09)

[1] CI = Confidence Interval.

[2] Predicted Probabilities are based on a cow from the average farm and are representative of the parity and calving date structure in the data.

[a,b] Predicted probabilities within a column with different superscripts differ ($P < 0.05$).

[‡] Interaction between synchronization treatment and calving group ($P = 0.13$).