Ovsynch versus Ultrasynch: Reproductive efficacy of a dairy cattle synchronization protocol incorporating corpus luteum function

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ABSTRACT

The objective was to compare the reproductive efficacy of Ultrasynch, a synchronization program based on functionality of the corpus luteum as determined by ultrasonography, with an Ovsynch protocol. A randomized field trial was conducted on a commercial dairy in Cayuga County, New York, during scheduled weekly pregnancy examinations. Cows ($n = 745$) determined nonpregnant 28 to 34 d after artificial insemination (AI) were randomly assigned to Ultrasynch or Ovsynch protocols. Cows assigned to the Ultrasynch management program ($n = 366$) were treated based on corpus luteum (CL) diameter: cows with a CL >23 mm received an injection of PGF_{2 α} and were bred via AI following detection of estrus (Ultra-PGF), whereas cows with a CL ≤ 23 mm received injections and were bred on an Ovsynch protocol. Cows assigned to the Ovsynch management program ($n = 379$) were placed on an Ovsynch protocol regardless of CL diameter. Pregnancy status was rechecked 28 to 34 d after AI; cows determined nonpregnant after initial enrollment were maintained in their assigned management group and received treatments based on CL diameter if in the Ultrasynch group and Ovsynch treatments if in the Ovsynch group. Hazard of pregnancy was similar between Ultrasynch and Ovsynch (hazard ratio = 1.10, 95% confidence interval = 0.88–1.36). Median days to conception were 98 and 87 for Ultrasynch and Ovsynch, respectively. The detection of estrus rate of cows in the Ultra-PGF group was 49%; better performance of an Ultrasynch management program may be achievable in a herd with a higher rate of estrus detection.

Key words: dairy cow, reproduction, synchronization, ultrasound

INTRODUCTION

Profitability of dairy farms depends on many factors, one of which is the reproductive efficiency of dairy

cows (Britt, 1985; Plaizier et al., 1997; Meadows et al., 2005). There is a large cost associated with the number of days open (Groenendaal et al., 2004; De Vries, 2006), and this reproductive failure is an important cause of economic loss for dairy farmers (Plaizier et al., 1997). Unfortunately, reproductive efficiency has declined for dairy cows worldwide and has been the trend for dairy fertility since 1957 (Lucy, 2007). A major component of this decline may be attributed to reduced estrus detection, anestrus, and reduced expression of estrus (Pursley et al., 1997; Lucy, 2001; Bicalho et al., 2007); these factors represent one of the major challenges in reproductive management (Lopez et al., 2004).

To reduce dependency on the detection of visible signs of estrus, ovulation synchronization protocols were developed to allow cows to be inseminated at a fixed time; the use of these protocols has become common practice in the dairy industry (Caraviello et al., 2006). The Ovsynch protocol (Pursley et al., 1995) is the most commonly used timed AI (TAI) program in dairy herds; however, treatments based on the presence or absence of a corpus luteum (CL) are still common practice (McDougall and Rhodes, 1999; McDougall, 2003; Bartolome et al., 2005). An accurate diagnosis of a CL becomes paramount for successful treatment with PGF_{2 α} , because this drug is most effective when an active CL is present. Previous research by our group showed that using a threshold diameter of 23 mm greatly increased accuracy of active CL detection by ultrasonography (Bicalho et al., 2008).

Conception to an Ovsynch protocol was markedly decreased during resynchronization compared with first insemination (Fricke et al., 2003) mainly because of a lack of presynchronization (Moreira et al., 2001; Galvão et al., 2007). Therefore, in herds with good detection of estrus, it is possible that selectively administering PGF_{2 α} to cows with an active CL may result in a similar time to conception compared with enrollment of all cows to Ovsynch. Such an approach, if successful, would be economically advantageous because of a saving in hormone injection costs.

The primary objective was to compare the reproductive efficiency of Ultrasynch, an estrus synchronization program that bases hormonal treatments on the pres-

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ence or absence of an active CL, to a standard Ovsynch program.

MATERIALS AND METHODS

Farm and Management

Data were collected from a dairy farm in Cayuga County, New York, from October 6, 2008, until March 2, 2009. This farm was selected because of its long-standing relationship with Cornell University's Ambulatory and Production Medicine Clinic. The farm milked 2,750 Holstein cows 3 times daily averaging 40.9 ± 0.4 kg of milk per cow per day during the study period. Cows were housed in freestall barns with concrete stalls covered with mattresses and bedded with recycled manure solids. Cows were fed a TMR consisting of approximately 55% forage (corn silage, alfalfa and grass haylage, alfalfa silage, alfalfa hay, and wheat straw) and 45% concentrate (cornmeal, soybean meal, canola meal, cottonseed, and citrus pulp). The diet was formulated to meet or exceed the NRC (2001) requirements for lactating Holstein cows weighing 650 kg and producing 40 kg of 3.7% FCM.

The prestudy reproductive management used a combination of Presynch (Moreira et al., 2001), Ovsynch (Pursley et al., 1995), Resynch (Fricke et al., 2003), and detection of estrus, with 25 to 30% of cows bred via TAI and the remainder bred after detection of estrus solely by using activity monitors (Alpro, DeLaval, Kansas City, MO). Estrus detection was based on electronic activity sensors worn around the neck; no cows were AI based on visual detection of estrus. Activity sensors determined the level of activity in a cow, and the computer compared it to her rolling activity over the previous week. Cows were then classified as having an activity of 0, 1, 2, or 3 and AI when they attained an activity level of 3. Inseminations were conducted once daily. Cows bred via TAI were separated by automatic sort gates during the first milking of the day for breeding, and cows that triggered the estrus activity monitoring system were AI after the milking following detection of estrus. Veterinarians from the Cornell Ambulatory and Production Medicine Clinic administered the necessary hormone injections to cows determined nonpregnant at palpation; all other reproductive injections, as well as all AI, were executed by 3 trained farm personnel. First-service conception rate for cows during the trial was 41% based on ultrasonography at 28 to 34 d post-AI (DPAI).

Data Collection and Study Design

A randomized field trial was designed involving cows in all stages of lactation. Before the beginning of the

trial, all cows were randomly assigned to 1 of 2 synchronization management groups: Ovsynch (control) and Ultrasynch (treatment). Randomization was completed in Excel (Microsoft, Redmond, WA) using the random number function and imported into the farm's Dairy Comp 305 program (Valley Agricultural Software, Tulare, CA).

During weekly pregnancy checks, cows 28 to 34 DPAI were separated from their penmates using an automatic sort gate, restrained by head locks, and examined by transrectal ultrasonography for pregnancy diagnosis and measurement of CL diameter. Ultrasonography of the CL and conceptus was performed according to Fricke (2002) and accomplished using 2 ultrasound units: an Easi-Scan portable bovine ultrasound with a 5-MHz linear probe and 7.5×10 cm wrist-mounted LCD display (Steuart Medical, Rochester, MN) and an Ultra Scan 50 portable front harness ultrasound with a 5-MHz linear probe and 7×10 cm display (Alliance Medical Inc., St. Joseph, MO). Ultrasonography was carried out by 1 of 3 trained researchers associated with the Cornell University Ambulatory and Production Medicine Clinic: a faculty member, a resident, and a postdoctoral veterinarian.

Cows determined nonpregnant at pregnancy examination were enrolled based on their previous random assignment; nonpregnant cows placed on the do-not-breed list were excluded. Cows assigned to the control group were placed on an Ovsynch protocol; an initial injection of 100 μ g of GnRH (Factrel, Fort Dodge Animal Health, Fort Dodge, IA) was given at pregnancy examination, followed 7 d later with 25 mg of PGF_{2 α} (Lutalyse, Pfizer, New York, NY). Forty-eight hours later, cows received a second injection of 100 μ g of GnRH and were AI via TAI 20 to 24 h later. Cows detected in estrus by the activity monitoring system were AI and removed from the TAI list. Cows determined nonpregnant after initial enrollment in the Ovsynch management group were again enrolled on an Ovsynch program.

Cows assigned to the treatment group were placed on an Ultrasynch protocol and were treated based on the largest diameter of any detectable CL: cows with CL >23 mm (**Ultra-PGF**) were given an injection of 25 mg of PGF_{2 α} and AI following detection of estrus during the following 2 wk, and cows with CL ≤ 23 mm received injections and were bred on an Ovsynch protocol (**Ultra-Ov**). If estrus was not detected in an Ultra-PGF cow within 14 d of PGF_{2 α} administration, the cow was examined at the following herd check and enrolled in the appropriate Ultra-PGF or Ultra-Ov group based on CL diameter. Cows determined nonpregnant after initial insemination in the Ultrasynch management group were again enrolled to the appropriate Ultra-PGF

Figure 1. Flowchart showing assignment of cows to treatment groups (n), synchronization protocol, and breeding method. DPAI = days post-AI; P4 = progesterone; CL = corpus luteum.

or Ultra-Ov group based on CL diameter. The Ultrasynch management system was specifically designed to allow cows to oscillate between Ultra-PGF or Ultra-Ov subgroups during the trial. A flowchart showing cow assignment to treatment groups is provided in Figure 1.

Additional data were collected on BCS, CL diameter, and DIM at enrollment as well as lactation number (1, 2, or ≥ 3). Group allocation, pregnancy diagnosis, and administration of injections on the day of pregnancy examination were completed by the aforementioned veterinarians, who were not blinded to group assignment. All other hormone injections, TAI, and AI following detection of estrus were performed by trained farm personnel unaware of group assignment.

Blood samples were collected from all study cows to replicate previous results associating CL diameter with blood progesterone level. Using a Vacutainer tube without anticoagulant and a 20 gauge $\times$ 2.54 cm Vacutainer needle (Becton, Dickinson and Company, Franklin Lakes, NJ), 10 mL of blood was collected from the coccygeal vein or artery of each cow. After collection of all blood samples on a given day, the samples were transported to the laboratory in ambient temperatures, spun

in a centrifuge at $2,000 \times g$ for 15 min at 4°C , and the serum was removed and frozen at -25°C . Circulating concentrations of progesterone were determined in 7 assays using a solid-phase radioimmunoassay kit containing antibody-coated tubes and ^{125}I -labeled progesterone (Coat-a-Count, Diagnostic Products Corporation, Los Angeles, CA) according to the manufacturer's instructions. The assay sensitivity was 0.01 ng/mL, and the mean intraassay and interassay coefficients of variation were 4.48 and 8.65%, respectively.

The study aimed to enroll at least 800 cows determined nonpregnant at 28 to 34 DPAI. This sample size, assuming a desired type I error rate of 5%, a power of 80%, and a baseline probability of conception of 0.35, would allow detection differences in the probability of conception between the 2 treatment groups of 0.1. A proposal was reviewed and approved by the Cornell University Institutional Animal Care and Use Committee (#2009-0001).

Statistical Analysis

Descriptive statistics were generated with the UNIVARIATE, MEANS, and FREQ procedures of SAS

Table 1. Descriptive statistics ($\pm$ SE) of treatment groups at study enrollment; the study enrolled 379 cows in the Ovsynch group and 366 cows in the Ultrasynch group¹

Treatment	LACT	DIM	BCS	CL (cm)
Ovsynch	2.04 $\pm$ 0.06	250 $\pm$ 4.9	3.2 $\pm$ 0.02	1.85 $\pm$ 0.07
Ultrasynch	2.08 $\pm$ 0.06	252 $\pm$ 5.6	3.2 $\pm$ 0.02	1.84 $\pm$ 0.07

¹No difference was found between the Ovsynch and Ultrasynch group in lactation number (LACT), DIM, BCS, or corpus luteum (CL) diameter.

(SAS Inst. Inc., Cary, NC). The CL diameter at which sensitivity and specificity were optimized for ultrasonographic detection was evaluated by receiver operating characteristic (ROC) curve analysis using MedCalc version 9.2.0.1 (MedCalc Software, Mariakerke, Belgium). The ROC curve analysis plots the sensitivity against the false positive fraction (1 – specificity) to detect the best combination of sensitivity and specificity for all possible CL diameters. The point closest to the upper left corner of the ROC curve represents the best combination of sensitivity and specificity and is selected automatically by the MedCalc software.

The effect of treatment group on the time from enrollment to conception was analyzed by Cox's proportional hazards. The time series variable for the model was enrollment-to-conception interval or enrollment-to-censor interval. A censoring variable was used to detect cows pregnant by the end the study from those that either died (or were culled) or were simply not detected pregnant by the end of the study. The Cox's proportional hazards model was fitted using the TPHREG procedure of SAS. Furthermore, potential confounding variables (such as age, parity, milk production, season at calving, caving ease score, and others) were extracted from Dairy Comp 305 management software and offered to the model as independent variables. Variables and their respective interaction terms were considered significant when $P \leq 0.05$. Independent variables were manually removed stepwise from the model by descending order of P -value.

RESULTS

Descriptive Statistics

A total of 745 cows were used in the analysis, 379 in the Ovsynch and 366 in the Ultrasynch group. A cow could be enrolled multiple times if she was continuously found nonpregnant at pregnancy examination; thus, there were 1,242 total enrollments, 583 in the Ovsynch group and 660 in the Ultrasynch group. Of enrollments in the Ultrasynch group, 43% had a CL diameter >23 mm (Ultra-PGF) and 57% had a CL diameter $\leq$ 23 mm (Ultra-Ov). Descriptive measures of the 2 treatment groups concerning BCS, CL diameter, and average

DIM at enrollment as well as lactation group are in Table 1, which shows effective randomization to treatment groups.

The conception risk for each level of the variables BCS, CL size, and serum progesterone concentration did not differ between the Ultrasynch and Ovsynch groups. Additionally, differences in BCS, CL size, and serum progesterone concentration at enrollment did not have an effect on conception risk within treatment group. Detection of estrus of Ultra-PGF cows did not differ based on BCS, CL size, or serum progesterone concentration at enrollment.

Detection of Estrus

Of the 287 Ultra-PGF enrollments, 141 (49%) were detected in estrus; distribution of days until detection of estrus from PGF_{2 α} administration can be seen in Figure 2. Detection of estrus of Ultra-PGF enrollments did not differ based on BCS, CL size, or blood progesterone level, nor was there any difference in estrus detection based on DPAI at enrollment.

Serum Progesterone Level and CL Diameter

A ROC curve analysis was conducted on serum blood progesterone levels to verify a minimum CL diameter threshold as performed by Bicalho et al. (2008). The sensitivity and specificity of an active CL via ultrasound, identified by a concurrent serum progesterone level of ≥ 1 ng/mL, was optimized using a threshold diameter of 20 mm (Figure 3).

Effect of Treatment on Reproductive Performance

A Cox's proportional hazards analysis showed that the hazard of pregnancy was similar between cows in the Ovsynch and Ultrasynch groups, with Ovsynch cows 1.10 (95% CI = 0.88–1.36) times more likely to conceive than cows in the Ultrasynch group ($P = 0.40$; Table 2). All confounding variables offered to the model were nonsignificant and excluded from the model. To control for the known effect of parity on reproductive performance, the independent variable parity number was forced into the Cox's proportional hazard model;

Figure 2. Histogram showing the number of days to detection of estrus post-PGF_{2α} administration of the 141 (49%) Ultrasynch cows with a corpus luteum >23 mm in diameter detected in estrus (Ultra-PGF). Maximum detection of estrus occurred at 4 d post-PGF_{2α} with 48 (34%) cows in estrus.

there was no significant difference between the 3 parity groups ($P = 0.31$).

To illustrate the hazard of pregnancy over time, a Kaplan-Meier survival curve analysis was performed. There was no difference in median days to conception between cows in the Ovsynch or Ultrasynch groups at 87 and 98 d, respectively ($P = 0.40$; Figure 4).

DISCUSSION

The study was conducted to determine how the reproductive efficacy of Ultrasynch, a synchronization program based on functionality of the CL, compared with that of a standard Ovsynch protocol. Analysis of the results showed no significant difference in conception rate or median days from enrollment to conception between groups.

Few studies have investigated conception after first-service AI based on CL diameter and detection of estrus. A trial completed by López-Gatius et al. (2004) compared first- and second-service AI pregnancy rates of cows on Ovsynch with cows in specific synchronization groups (CL diameter ≥ 15 mm, natural estrus, anovulatory follicle, and ovarian cyst). Preinsemination treatments were based on observed ovarian structures present at protocol initiation. By assigning cows to specific synchronization groups, they were successful in significantly improving pregnancy rates over the control Ovsynch group for both first-service AI and cumulative second-service AI. Yet, their study did not examine how cows in specific synchronization groups performed after multiple services, whereas the present study observed conception in cows found nonpregnant to at least one service.

Although our analysis conclusively determined there was no difference in time to conception between groups, 2 issues must be explored to account for the less-than-hypothesized conception results from the Ultrasynch group: detection of estrus and assignment to Ultrasynch subgroup based on a CL threshold diameter of 23 mm. Cows in the Ultrasynch treatment group may not have conceived sooner than cows in the Ovsynch control group because of Ultrasynch's reliance on detection of estrus. Considering that 43% of enrollments in the treatment group were bred solely on estrus, the on-farm rate of detection of estrus played a large role in the time to conception of Ultrasynch enrollees. The study herd estrus detection rate of Ultra-PGF cows was 49%, much lower than values found in other studies (Stevenson et al., 2003; Cairoli et al., 2006).

Stevenson et al. (2003) examined the effects of PGF_{2α} and estrus detection on cows found nonpregnant at first pregnancy examination. Cows given PGF_{2α} at nonpregnant diagnosis and AI after detected estrus (or 72 to 80 h after PGF_{2α} treatment if no estrus was detected) had similar conception rates compared with cows AI solely off estrus, at 22 and 23%, respectively. Estrus, found by visual observation and tail chalk evaluation after

Figure 3. Receiver operating characteristic curve analysis of blood progesterone level and corpus luteum (CL) diameter of 1,242 cow enrollments after nonpregnant diagnosis at 28 to 34 d post-AI. The sensitivity and specificity of an active CL via ultrasound, identified by concurrent serum progesterone level of ≥ 1 ng/mL, was optimized using a threshold diameter of 20 mm (sensitivity = 80.0, specificity = 72.0). The area under the curve was 0.82 (95% CI = 0.80–0.84).

Figure 4. Kaplan-Meier survival analysis illustrating the effect of synchronization protocol on time to conception for 379 cows enrolled in Ovsynch and 366 cows enrolled in Ultrasynch. There was no difference in median days to conception between cows in the Ovsynch or Ultrasynch groups at 87 and 98 d, respectively ($P = 0.40$).

morning milking, was detected in 75% of cows given PGF_{2α} at nonpregnant diagnosis. Similarly, Cairoli et al. (2006) observed estrus within 7 d of PGF_{2α} administration in 82% (230/280) of cows diagnosed with a CL via rectal palpation.

It is unclear why the estrus detection rate in the study was low; analysis of the herd's Dairy Comp 305 records for the 6 mo following completion of the study showed an estrus detection rate of 60%, calculated by examining the number of cows found nonpregnant at pregnancy exam that did not have an estrus detection event since the previous breeding. During the trial, approximately 300 of the activity transponders were replaced because they were no longer transmitting activity data to the monitoring system. It is unknown

whether the 300 cows with replaced activity transponders were enrolled, and if so, to which group they may have been assigned. This technical failure likely had an effect on the time to conception for cows in both treatment groups, especially the Ultra-PGF cows because they were being AI solely on estrus detection. Although the rate of detection of estrus was lower than that found in other studies, time to estrus detection post-PGF_{2α} administration was similar. In Cairoli et al. (2006), 95% of the cows found in estrus after receiving PGF_{2α} were detected between 3 and 5 d post-PGF_{2α} administration, with the majority detected on d 4 (57%). Other studies have produced supporting results (Archbald et al., 1993; Stevens et al., 1993). It is difficult to model the outcome of Ultrasynch using a higher rate of detection

Table 2. Cox's proportional hazard ratio showing the effect of treatment group and lactation on median days to conception

Item	Median days to conception	Pregnant (%)	Parameter estimate (SE)	Hazard ratio	P-value
Treatment					
Ovsynch	87	48.8 (n = 379)	0.09 (0.11) Baseline	1.10	0.40
Ultrasynch	98	45.1 (n = 366)			
Lactation					
1	89	52.1 (n = 286)	0.21 (0.14)	1.24	0.31
2	87	46.7 (n = 259)	0.15 (0.14)	1.17	
≥3	97	40.0 (n = 200)	Baseline		

of estrus because of study design. Use of the program in a herd with better estrus detection would, in theory, produce superior results.

Another possible effect on time to conception for cows in the Ultrasynch group was their subgroup assignment to Ultra-PGF or Ultra-Ov based on presence of a CL with diameter >23 or ≤ 23 mm. These assignments were based on data from Bicalho et al. (2008), which analyzed blood progesterone concentrations and CL diameter in cows 21 and 24 d post-AI and found the optimal thresholds by ROC curve analysis to be 23 mm and 22 mm, respectively. The cows in this study had their CL diameter and blood progesterone measurements taken at 28 to 34 DPAI, at which point ROC curve analysis found the optimal threshold measurement of 20 mm. Had the study assigned Ultrasynch cows to Ultra-PGF or Ultra-Ov based on a 20-mm threshold diameter, a higher percentage of cows would have been assigned to Ultra-PGF. In the study herd, this would most likely have increased the median days to conception for Ultrasynch cows. As mentioned above, median days to conception may decrease compared with Ovsynch when using a 20-mm threshold diameter in a herd with a higher rate of detection of estrus.

Differences in conception data for Ultra-PGF and Ultra-Ov cows cannot be interpreted because of the study design. Cows enrolled in Ultra-PGF and that were not detected in estrus within 14 d or did not become pregnant were reassigned to the appropriate Ultrasynch subgroup based on CL diameter. Thus, a cow could oscillate between Ultra-PGF and Ultra-Ov multiple times during the trial if she was found nonpregnant repeatedly or was not detected in estrus. Because of this potential switching between subgroups by any given cow, a time at risk cannot be established and the 2 subgroups cannot be compared with each other or compared individually with cows in the Ovsynch group.

Economically, the Ultrasynch management program was less expensive than the Ovsynch program. With a herd estrus detection rate of 49%, cows in the Ultrasynch group were palpated a total of 659 times and received 659 PGF_{2α} and 748 GnRH injections; cows in the Ovsynch group were palpated a total of 586 times and received 586 PGF_{2α} and 1,172 GnRH injections. Use of Ultrasynch in a herd with a greater estrus detection rate would result in a shorter time to conception for cows in the Ultra-PGF group, decreasing the need for repeated palpations and hormonal injections and further decreasing reproductive management costs.

These observations suggest that the use of a synchronization program based on functionality of the CL, defined by a threshold diameter of 23 mm, was as effective as a traditional Ovsynch program. In herds with estrus detection $>50\%$, the Ultrasynch management

program may be a viable alternative to Ovsynch, with an additional benefit of having lower costs. However, the use of a threshold diameter of 20 mm may provide different results. In addition, better performance of such a program may be achievable on a herd with a high rate of estrus detection.

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