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## Resynchronization of ovulation and timed insemination in lactating dairy cows, II: assigning protocols according to stages of the estrous cycle, or presence of ovarian cysts or anestrus

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### Abstract

Pregnancy rates were compared in lactating dairy cows ( $n = 1083$ ) assigned to protocols for resynchronization of ovulation based on stages of the estrous cycle, or presence of ovarian cysts or anestrus. Cows were detected not pregnant by ultrasonography 30 d after a previous AI (study day 0) and classified as diestrus, metestrus, proestrus, with ovarian cysts or anestrus. Cows in diestrus (January–May) were assigned to either Ovsynch (GnRH day 0, PGF<sub>2α</sub> day 7, GnRH day 9, and timed-AI [TAI] 16 h later;  $n = 96$ ), or Quicksynch (PGF<sub>2α</sub> day 0, estradiol cypionate [ECP] day 1, AI at detected estrus [AIDE] on day 2, or TAI on day 3;  $n = 96$ ). Cows in diestrus (June–December) were assigned to either Ovsynch ( $n = 156$ ) or Modified Quicksynch (PGF<sub>2α</sub> day 0, ECP day 1, AIDE days 2 and 3, and to Ovsynch on day 4 if not detected in estrus;  $n = 142$ ). Cows in metestrus were assigned either to Ovsynch ( $n = 68$ ), Heatsynch (GnRH day 0, PGF<sub>2α</sub> day 7, ECP day 8, AIDE day 9, or TAI day 10;  $n = 62$ ), or GnRH + Ovsynch (GnRH on day 0, followed by Ovsynch on day 8;  $n = 64$ ). Cows

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in proestrus, with ovarian cysts, or anestrus were assigned to either Ovsynch (proestrus  $n = 89$ , ovarian cysts  $n = 97$ , anestrus  $n = 8$ ) or GnRH + Ovsynch (proestrus  $n = 87$ , ovarian cysts  $n = 109$ , anestrus  $n = 9$ ). Pregnancy rate was evaluated 30, 55 and 90 d after resynchronized AI. For cows in diestrus (January–May), pregnancy rates were higher for Ovsynch (35.9, 29.2 and 26.0%) than for Quicksynch (21.7, 16.7 and 15.6%). For cows in diestrus (June–December), pregnancy rates were similar for Ovsynch (34.4, 24.0 and 23.6%) and Modified Quicksynch (27.1, 26.2 and 21.6%). For cows in metestrus, pregnancy rates were higher for GnRH + Ovsynch (33.3, 24.5 and 20.3%) than for Heatsynch (20.3, 12.9 and 9.8%). For cows with ovarian cysts, pregnancy rates were higher for GnRH + Ovsynch (30.3, 26.6 and 22.9%) than for Ovsynch (20.2, 18.5 and 14.7%). Assignment to resynchronization protocols based on the stages of the estrous cycle, or presence of ovarian cysts improved pregnancy rates.

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*Keywords:* Resynchronization; Timed-AI; Dairy cows; Estrous cycle

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## 1. Introduction

Synchronization of ovulation and timed-AI (TAI) increases the number of cows inseminated after the voluntary waiting period and consequently increases pregnancy rate [1–7]. Protocols for TAI are more efficient when initiated at a particular stage of the estrous cycle [8,9]. After first services, non-pregnant cows need to be reinseminated in a timely manner to maintain reproductive efficiency [10]. Therefore, protocols for resynchronization of ovulation and TAI need to be short, but still consider the stage of the estrous cycle.

Per rectum examination and ultrasonography of the genital tract were used to determine stages of the estrous cycle, presence of ovarian cysts or anestrus which influenced the response to the Ovsynch and Heatsynch protocols for resynchronization [11]. Therefore, pregnancy rates could be optimized by assigning different resynchronization of ovulation protocols according to stages of the estrous cycle, or presence of ovarian cysts or anestrus.

The objective of this study was to compare pregnancy rates at days 30, 55 and 90 and pregnancy losses between days 30 and 55 and days 55 and 90 in lactating dairy cows subjected to different protocols of resynchronization of ovulation for TAI. These protocols were assigned based on stages of the estrous cycle, or presence of ovarian cysts or anestrus at the time of non-pregnancy diagnosis by ultrasonography.

## 2. Materials and methods

### 2.1. Study population

The study was conducted from January to December 2002 in a large commercial dairy in north central Florida with 3200 milking cows. The herd is divided into 14 lots according to levels of production and stage of lactation; cows from all lots were included in the study. Cows were housed in free stall barns and dry lots and fed a total mixed ration (TMR) thrice daily. The TMR was formulated to meet or exceed requirements for lactation [12]. Cows were milked three times a day and had a rolling herd average for milk production of

10,700 kg. Beginning at 60 d postpartum, cows received a bST treatment (Posilac<sup>®</sup>, 500 mg sometribove zinc, subcutaneously; Monsanto, St. Louis, MO, USA) every 14 d for the remaining lactation. Reproductive management consisted of a voluntary waiting period of 75 d that incorporated a Presynch-Ovsynch program [13] for first service, and estrus detection using visual observation and a computerized pedometer system (Afimilk<sup>®</sup>, S.A.E. Afikim; Kibbutz Afikim, Israel) for subsequent services. The study included 1083 lactating dairy cows determined to be non-pregnant by ultrasonography at day 30 (study day 0) after AI. Cows with reproductive abnormalities (i.e. metritis, pyometra, uterine-ovarian adhesions) were not included in the study.

## 2.2. Study design

On day 0, cows were classified into different stages of the estrous cycle or with ovarian cysts or anestrus as determined by per rectum examination and ultrasonography of the genital tract [11] and then assigned to various resynchronization of ovulation for TAI protocols (Fig. 1).

The distribution of cows at different stages of the estrous cycle or with ovarian cysts or anestrus on day 0 ( $n = 1083$ ) was as follows: diestrus 45.2%, metestrus 17.9%, proestrus 16.2%, ovarian cysts 19.0%, and anestrus 1.6%.

Cows in diestrus from January to May ( $n = 192$ ) were randomly divided into two groups: Ovsynch Group ( $n = 96$ ) and Quicksynch Group ( $n = 96$ ). Cows in diestrus from June to December ( $n = 298$ ) were randomly divided into two groups: Ovsynch Group ( $n = 156$ ) and Modified Quicksynch Group ( $n = 142$ ). Cows in metestrus from January to December were randomly divided into three groups: Ovsynch Group ( $n = 68$ ), Heatsynch Group ( $n = 62$ ), and GnRH+Ovsynch Group ( $n = 64$ ). Cows in proestrus, or with ovarian cysts, or anestrus from January to December were randomly divided into two groups: Ovsynch Group

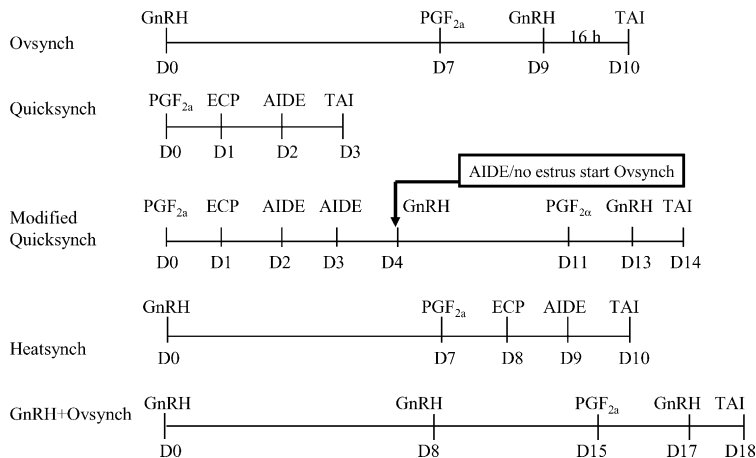


Fig. 1. Protocols used for resynchronization of ovulation and TAI depending upon stage of the estrous cycle, or ovarian cysts or anestrus at the time of a non-pregnant diagnosis at day 30 after AI. AIDE = AI at detected estrus based on visual observation and pedometers.

(proestrus  $n = 89$ , ovarian cysts  $n = 97$ , anestrus  $n = 8$ ) and GnRH + Ovsynch Group (proestrus  $n = 87$ , ovarian cysts  $n = 109$ , anestrus  $n = 9$ ). The hormonal doses utilized were 100  $\mu\text{g}$  i.m. of GnRH (2 mL of Cystorelin<sup>®</sup>; Merial Limited, Iselin, NJ, USA), 25 mg im of PGF<sub>2 $\alpha$</sub>  (5 mL of Lutalyse<sup>®</sup> Sterile Solution; Pfizer Animal Health, New York, NY, USA) and 1 mg im of ECP (0.5 mL of ECP<sup>®</sup> Sterile Suspension; Pfizer Animal Health). Cows were TAI 16 h after GnRH and 48 h after ECP. Cows detected in estrus between PGF<sub>2 $\alpha$</sub>  treatment and TAI were AI and included in the study. On day 0, parity, time of the year (season), days in milk (DIM), and inseminator were recorded.

### 2.3. Plasma progesterone ( $P_4$ ) concentration

On day 0, a blood sample was obtained from the coccygeal vessels into evacuated tubes containing EDTA (Vacutainer<sup>®</sup>; BD, Franklin Lakes, NJ, USA) and placed on ice immediately after collection. Samples were centrifuged at 3000  $\times$  g for 30 min, and plasma was stored at  $-20^\circ\text{C}$  until assayed for  $P_4$  using a solid-phase, no-extraction RIA (Coat-a-Count Progesterone, DPC<sup>®</sup>; Diagnostic Products Corporation, Los Angeles, CA, USA). The standard curve dilution consisted of four plain tubes for total counts and coated tubes for non-specific binding and 100  $\mu\text{L}$  of increasing concentrations of  $P_4$  (0.1, 0.25, 0.5, 2, 5, 10, 20, and 40 ng/mL). Serum from a diestrus cow was used as a reference (6.85 ng/mL). A duplicate was included every sixth sample. Sensitivity of the assay was 0.1 ng/mL. The duplicates were categorized into high ( $>3$  ng/mL), medium (1–3 ng/mL) and low ( $<1$  ng/mL) concentrations of  $P_4$ . The intra-assay coefficients of variation for the high, medium and low duplicates samples were 5.5, 4.8 and 9.3%, respectively. Intra and inter-assay coefficients of variation for the luteal phase reference sample were 12.5 and 10.5%, respectively.

Plasma  $P_4$  concentration was used as the reference to determine the positive predictive value of per rectum examination and ultrasonography of the genital tract to determine stages of the estrous cycle, or presence of ovarian cysts or anestrus. The limit values for plasma  $P_4$  concentration for different stages of the estrous cycle, or presence of ovarian cysts or anestrus were established as follow: diestrus  $>2$  ng/mL, metestrus  $<2$  ng/mL, proestrus  $<1$  ng/mL, anestrus  $<1$  ng/mL and ovarian cysts: follicular cysts  $<1$  ng/mL and luteal cysts  $> 1$  ng/mL [14]. Plasma  $P_4$  concentration also was used to determine sensitivity, specificity, and positive predicted value [15] of the clinical diagnosis (per rectum examination and ultrasonography of the genital tract) to differentiate follicular and luteal cysts [16].

### 2.4. Pregnancy diagnosis to resynchronized AI

Pregnancy diagnosis was conducted on day 30 ( $\pm 1$ ) after the resynchronized AI using an ultrasound scanner with a 5 MHz, linear-array transrectal transducer (Aloka 500 V; Wallingford, CT, USA). Cows were classified as: (1) pregnant: embryo proper with heartbeats and surrounded by a fluid-filled cavity representing the allantoic cavity detected in the uterine lumen [17,18]; (2) questionable pregnant: fluid in the uterine lumen and presence of a CL but no embryo observed, or (3) non-pregnant: cows that were AIDE before ultrasonography or non-pregnant at ultrasonography. On days 55 and 90, pregnancy

was reconfirmed by per rectum examination of the genital tract based on previous described criteria [19]. Among cows evaluated at day 55 and day 90 for pregnancy, the cows diagnosed non-pregnant at day 30 and day 55 were not palpated subsequently at day 55 and day 90, respectively. Cows classified as questionable pregnant were re-examined at day 55. The distribution of interestrus intervals was determined for cows that were diagnosed not pregnant based on AIDE prior to ultrasonography on day 30.

### 2.5. Statistical analysis

The distributions of cows by parity (1, 2, 3+), season, DIM (quartiles) and inseminator (A, B, C) among groups within each stage of the estrous cycle, or with ovarian cysts or anestrus were evaluated using chi-square test (PROC FREQ, SAS system). Pregnancy rates on days 30, 55 and 90 and pregnancy losses between days 30 and 55 and days 55 and 90 were evaluated in a multivariate analysis to adjust for variables that were not equally distributed among groups and to evaluate interactions. The model for each stage (i.e., stage of the estrous cycle, or ovarian cysts or anestrus) included treatment group, parity (1, 2, 3+), season, DIM (quartiles), inseminator (A, B, C) and interactions. The backward elimination method of the logistic regression procedure was used to obtain the final model (PROC GENMOD, SAS system; [20]). Variables remained in the model at  $P < 0.15$ . Plasma  $P_4$  concentrations were evaluated using the least squares means analysis of variance (PROC GLM, SAS system) and proportions of cows with low or high  $P_4$  using the Chi-square test (PROC FREQ, SAS system). Treatment differences with  $P \leq 0.05$  were considered significant, whereas tendencies were considered when  $P > 0.05$  and  $P \leq 0.15$ .

## 3. Results

The numbers of cows evaluated at days 30, 55 and 90 are described in Table 1. Overall pregnancy rates were 28% ( $n = 919$ ) on day 30, 23.1% ( $n = 1075$ ) on day 55, and 20.3% ( $n = 1065$ ) on day 90. Among the 72% (662/919) of cows detected non-pregnant on day 30 after resynchronized AI, 54% (359/662) were diagnosed not pregnant based on expression of estrus and AI before ultrasonography, and 46% (303/662) based on the absence of pregnancy at ultrasonography. The median interestrus interval for cows diagnosed not pregnant based on expression of estrus and AI before ultrasonography was 22 d, and the distribution of interestrus intervals is described in Fig. 2. Among the 359 cows, 13.7% showed estrus before 17 d, 64.3% showed estrus within 17–24 d, and 22.0% showed estrus 25 d after resynchronized AI.

### 3.1. Cows in diestrus (January–May)

The distributions of cows by parity ( $P = 0.18$ ), season ( $P = 0.19$ ), DIM ( $P = 0.26$ ) and inseminator ( $P = 0.48$ ) were not different between groups. Pregnancy rate on day 30 was higher ( $P < 0.05$ ) for cows in the Ovsynch Group (35.9%) compared to cows in the Quicksynch Group (21.7%). Pregnancy rate on days 55 and 90 tended ( $P < 0.07$ ) to be higher for cows in the Ovsynch Group (29.2 and 26.0%) compared to cows in the

Table 1

Number of cows evaluated for different stages of the estrous cycle, or with ovarian cysts or anestrus and in the protocols at day 30, 55 and 90 after resynchronized AI

Stage	Protocol	Day 30				Day 55			Day 90		
		N/U	Q/P	D	T	C	D	T	C	D	T
Diestrus	Ovsynch	2	2	92	96	–	96	96	–	96	96
(Jan–Mar)	Quicksynch	–	4	92	96	–	96	96	–	96	96
Diestrus	Ovsynch	28	–	128	156	2	154	156	1	153	154
(Jun–Dec)	M. Quicksynch	20	4	118	142	1	141	142	1	140	141
Metestrus	Ovsynch	9	1	58	68	–	68	68	–	68	68
	Heatsynch	8	–	54	62	–	62	62	1	61	62
	GnRH + Ovsynch	14	2	48	64	3	61	64	2	59	61
Proestrus	Ovsynch	15	1	73	89	1	88	89	1	87	61
	GnRH + Ovsynch	11	1	75	87	1	86	87	2	84	88
Cystic	Ovsynch	15	3	79	97	–	97	97	2	95	97
	GnRH + Ovsynch	19	1	89	109	–	109	109	–	109	109
Anestrus	Ovsynch	3	–	5	8	–	8	8	–	8	8
	GnRH + Ovsynch	1	–	8	9	–	9	9	–	9	9
Total		145	19	919	1083	8	1075	1083	10	1065	1075

N/U = not ultrasounded; Q/P = questionable pregnant; D = diagnosed; C = culled; T = total.

Quicksynch Group (16.7 and 15.6%). There was no difference in pregnancy losses between days 30–55 or days 55–90 for cows in the Ovsynch (21.2 and 10.7%) and Quicksynch (20.0 and 6.2%) groups (Table 2).

In the Quicksynch Group, 78% (72/92) of cows showed pedometer activity ± 1 d from TAI, and pregnancy rate on day 30 was higher ( $P < 0.01$ ) for the cows with pedometer activity (27.8%, 20/72) compared to cows without activity (0.0%, 0/20).

Plasma P<sub>4</sub> concentrations on day 0 were not different for cows in the Ovsynch (6.07 ± 0.32 ng/mL) and Quicksynch (6.41 ± 0.30 ng/mL) groups. There was no difference in the proportion of cows with plasma P<sub>4</sub> concentration greater than 2 ng/mL between Ovsynch (87.5%, 84/96) and Quicksynch (92.5%, 87/94) groups. The positive predictive value for per rectum examination and ultrasonography of the genital tract to detect cows in diestrus was 90% (171/190).

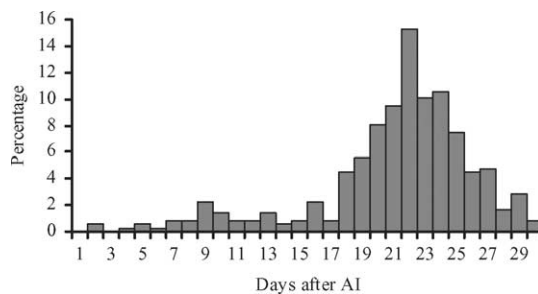


Fig. 2. Distribution of interestrus intervals in 359 lactating dairy cows diagnosed non-pregnant based on expression of estrus and insemination prior to ultrasonography for pregnancy diagnosis on day 30 after AI.

Table 2

Pregnancy rates at days 30, 55 and 90, and pregnancy losses between days 30 and 55 and days 55 and 90 for cows in diestrus for the Ovsynch and Quicksynch groups from January to May

Variable	Group	
	Ovsynch% (N)	Quicksynch% (N)
Pregnancy rate		
Day 30	35.9 (92) <sup>a</sup>	21.7 (92) <sup>a</sup>
Day 55	29.2 (96) <sup>b</sup>	16.7 (96) <sup>b</sup>
Day 90	26.0 (96) <sup>b</sup>	15.6 (96) <sup>b</sup>
Pregnancy losses		
Day 30–55	21.2 (33)	20.0 (20)
Day 55–90	10.7 (28)	6.2 (16)

<sup>a</sup> ( $P < 0.05$ ).

<sup>b</sup> ( $P < 0.07$ ).

### 3.2. Cows in diestrus (June–December)

The distributions of cows by parity ( $P = 0.53$ ) and season ( $P = 0.93$ ) between groups were not different, but tended to be different for DIM ( $P = 0.07$ ) and was different among inseminators ( $P < 0.01$ ). Pregnancy rates on days 30, 55, and 90 for cows in the Ovsynch (34.0, 24 and 23.6%) and Modified Quicksynch (27.1, 26.2, and 21.6%) groups were not different. Pregnancy losses between days 30 and 55 were higher ( $P < 0.05$ ) for cows in the Ovsynch Group (23.2%) compared to cows in the Modified Quicksynch Group (6.4%). Pregnancy losses between days 55 and 90 for cows in the Ovsynch (5.7%) and Modified Quicksynch (8.3%) groups were not different (Table 3).

In the Modified Quicksynch Group, 72.0% (85/118) of the cows were AIDE using pedometer activity and 28.0% (33/118) were TAI. Pregnancy rate on day 30 for cows AIDE (29.0%; 25/85) and for cows TAI (21.1%; 7/33) within the Modified Quicksynch Group were not different.

Plasma  $P_4$  concentrations on day 0 for cows in the Ovsynch ( $4.77 \pm 0.20$  ng/mL) and Modified Quicksynch ( $4.77 \pm 0.19$  ng/mL) groups were not different. There were no differences in the proportions of cows with plasma  $P_4$  concentration greater than 2 ng/mL

Table 3

Pregnancy rates at days 30, 55 and 90, and pregnancy losses between days 30, 55, 55 and 90 for cows in diestrus for the Ovsynch and Modified Quicksynch groups from June to December

Variable	Group	
	Ovsynch% (N)	Modified Quicksynch% (N)
Pregnancy rate		
Day 30	34.4 (128)	27.1 (118)
Day 55	24.0 (154)	26.2 (141)
Day 90	23.6 (153)	21.6 (140)
Pregnancy losses		
Day 30–55	23.2 (43) <sup>a</sup>	6.4 (31) <sup>a</sup>
Day 55–90	5.7 (35)	8.3 (36)

<sup>a</sup>  $P < 0.05$ .

between the Ovsynch (89.5%, 136/152) and Modified Quicksynch (92.5%, 124/140) groups. The positive predictive value for per rectum examination and ultrasonography of the genital tract to detect cows in diestrus was 85.6% (250/292).

### 3.3. Cows in metestrus

The distributions of cows by parity ( $P = 0.67$ ), season ( $P = 0.97$ ), and inseminator ( $P = 0.80$ ) were not different, but were different for DIM ( $P = 0.04$ ). Pregnancy rate on day 30 tended ( $P < 0.13$ ) to be higher for cows in the GnRH + Ovsynch Group (33.3%) compared to cows in the Ovsynch (24.1%) and Heatsynch (20.3%) groups. Pregnancy rate on days 55 and 90 tended ( $P < 0.10$ ) to be higher for cows in the GnRH + Ovsynch (24.5 and 20.3%) and Ovsynch (25.3 and 23.8%) groups compared to cows in the Heatsynch Group (12.9 and 9.8%). Pregnancy losses between days 30 and 55 and days 55 and 90 for cows in the Ovsynch (7.1 and 5.9%), Heatsynch (36.3 and 14.3%), and GnRH + Ovsynch (13.3 and 7.8%) groups were not different (Table 4).

In the Heatsynch Group, 16.0% (10/62) of the cows were AIDE and 40.0% (4/10) were pregnant at day 30. In the Ovsynch Group, 10.4% (7/67) cows were AIDE and 28.6% (2/7) were pregnant at day 30. In the GnRH+Ovsynch Group only one cow of 59 (1.7%) was AIDE and was diagnosed pregnant at day 30.

Plasma  $P_4$  concentrations on day 0 for cows in the Ovsynch ( $2.21 \pm 0.33$  ng/mL), Heatsynch ( $1.64 \pm 0.27$  ng/mL), or GnRH + Ovsynch ( $1.78 \pm 0.31$  ng/mL) groups were not different. There were no differences in the proportion of cows with plasma  $P_4$  concentration lower than 2.0 ng/mL among cows in the Ovsynch (62.9%, 42/67), Heatsynch (76.3%, 45/59), and GnRH + Ovsynch (74.6%, 47/63) groups. The positive predictive value for per rectum examination and ultrasonography of the genital tract to detect cows in metestrus was 70.1% (134/189).

### 3.4. Cows in proestrus

The distributions of cows by parity ( $P = 0.86$ ), season ( $P = 0.32$ ), and DIM (0.93) were not different, but were different among inseminators ( $P < 0.01$ ). Pregnancy rates on days

Table 4

Pregnancy rates at days 30, 55 and 90, and pregnancy losses between days 30 and 55 and days 55 and 90 for cows in metestrus for the Ovsynch, Heatsynch, and GnRH + Ovsynch groups from January to December

Variable	Group		
	Ovsynch% (N)	Heatsynch% (N)	GnRH + Ovsynch% (N)
Pregnancy rate			
Day 30	24.1 (58) <sup>a</sup>	20.3 (54) <sup>a</sup>	33.3 (48) <sup>a</sup>
Day 55	25.0 (68) <sup>a</sup>	12.9 (62) <sup>a</sup>	24.5 (59) <sup>a</sup>
Day 90	23.5 (68) <sup>a</sup>	9.8 (61) <sup>a</sup>	20.3 (59) <sup>a</sup>
Pregnancy losses			
Day 30–55	7.1 (14)	36.3 (11)	13.3 (15)
Day 55–90	5.9 (17)	14.3 (7)	7.8 (13)

<sup>a</sup>  $P < 0.13$ .

Table 5

Pregnancy rates at days 30, 55 and 90, and pregnancy losses between days 30, 55, 55 and 90 for cows in proestrus for the Ovsynch and GnRH + Ovsynch groups from January to December

Variable	Group	
	Ovsynch% (N)	GnRH + Ovsynch% (N)
Pregnancy rate		
Day 30	30.1 (73)	28.0 (75)
Day 55	23.9 (88)	23.2 (86)
Day 90	21.8 (87)	20.2 (84)
Pregnancy losses		
Day 30–55	19.0 (21)	15.0 (20)
Day 55–90	5.2 (19)	5.9 (17)

30, 55 and 90 for cows in the Ovsynch (30.1, 23.9, 21.8%) and GnRH + Ovsynch (28.0, 23.2, and 20.2%) groups were not different. Pregnancy losses between days 30 and 55 and days 55 and 90 for cows in the Ovsynch (19.0 and 5.2%) and GnRH + Ovsynch (15.0 and 5.9%) groups were not different (Table 5).

Plasma P<sub>4</sub> concentrations on day 0 for cows in the Ovsynch (0.69 ± 0.13 ng/mL) and GnRH + Ovsynch (0.47 ± 0.09 ng/mL) groups were not different. There was no difference in the proportion of cows with plasma P<sub>4</sub> concentration lower than 1.0 ng/mL in the Ovsynch (80.0%, 70/87) and GnRH + Ovsynch (88.0%, 76/86) groups. The positive predictive value for per rectum examination and ultrasonography of the genital tract to detect cows in proestrus was 84.4% (146/173).

### 3.5. Cows with ovarian cysts

The distributions of cows by parity ( $P = 0.58$ ), season ( $P = 0.45$ ), and DIM (0.17) were not different, but were different among inseminators ( $P < 0.01$ ). Pregnancy rates on days 30, 55 and 90 for cows with ovarian cysts in the Ovsynch Group (20.2, 18.5, and 14.7%) were lower ( $P < 0.05$ ) compared to cows in the GnRH + Ovsynch Group (30.3, 26.6, and 22.9%). Pregnancy losses between days 30 and 55 and days 55 and 90 for cows in the Ovsynch (12.5 and 12.5%) and GnRH + Ovsynch (11.1 and 13.8%) groups were not different (Table 6).

Plasma P<sub>4</sub> concentrations on day 0 for cows with ovarian cysts in the Ovsynch (0.82 ± 0.10 ng/mL) and the GnRH + Ovsynch (0.87 ± 0.13 ng/mL) groups were not different. The proportions of cystic cows with follicular cysts determined by per rectum palpation and ultrasonography of the genital tract in the Ovsynch (89.7%, 87/97) and GnRH + Ovsynch (91.7%, 100/109) groups were not different. The proportion of cystic cows with plasma P<sub>4</sub> concentration lower than 1 ng/mL (follicular cysts) in the Ovsynch (72.3%, 68/94) and the GnRH + Ovsynch (73.5%, 78/106) were not different. Cows that underwent both diagnostic methods (clinical diagnosis using palpation per rectum and ultrasonography, and plasma P<sub>4</sub> concentration) were used to calculate sensitivity, specificity and positive predictive value of the clinical diagnosis to differentiate follicular and luteal cysts utilizing plasma P<sub>4</sub> as the gold standard. For cows clinically classified as presenting ovarian follicular cysts ( $n = 181$ ), 144 cows had plasma P<sub>4</sub> concentration lower than 1 ng/mL. For

Table 6

Pregnancy rates at days 30, 55 and 90, and pregnancy losses between days 30, 55, 55 and 90 for cows with ovarian cysts subjected to the Ovsynch and GnRH + Ovsynch groups from January to December

Variable	Group	
	Ovsynch% (N)	GnRH + Ovsynch% (N)
Pregnancy rate		
Day 30	20.2 (79) <sup>a</sup>	30.3 (89) <sup>a</sup>
Day 55	18.5 (97) <sup>a</sup>	26.6 (109) <sup>a</sup>
Day 90	14.7 (95) <sup>a</sup>	22.9 (109) <sup>a</sup>
Pregnancy losses		
Day 30–55	12.5 (16)	11.1 (27)
Day 55–90	12.5 (16)	13.8 (29)

<sup>a</sup>  $P < 0.05$ .

cows clinically classified as presenting ovarian luteal cysts ( $n = 19$ ), 17 cows had plasma  $P_4$  concentration greater than 1 ng/mL. The sensitivity, specificity and positive predictive value for per rectum examination in combination with ultrasonography to detect luteal and follicular cysts were 31.5, 98.6 and 89.5%, and 98.6, 31.5 and 79.6%, respectively.

There was an interaction ( $P < 0.05$ ) between group and type of ovarian cysts. Pregnancy rates on day 30, 55 and 90 in cows with follicular cysts as determined by clinical diagnosis was lower for the Ovsynch Group (18.6, 17.2 and 12.9%) compared to the GnRH + Ovsynch Group (31.1, 27.0 and 23.0%) but there was no differences for cows with luteal cysts. However, even though pregnancy rate was numerically higher for the GnRH + Ovsynch Group, there was no statistical difference between Ovsynch and GnRH + Ovsynch in cows with either low ( $< 1$  ng/mL) or high ( $> 1$  ng/mL) plasma  $P_4$  concentration (Table 7).

Table 7

Pregnancy rates (PR) at days 30, 55 and 90 for Ovsynch and GnRH + Ovsynch groups in cows with ovarian cysts classified according to type of cyst and plasma progesterone ( $P_4$ ) concentration

Variable	PR at day 30 % (N)	PR at day 55 % (N)	PR at day 90 % (N)
Follicular cyst			
Ovsynch	18.6 <sup>a</sup> (70)	17.2 <sup>a</sup> (87)	12.9 <sup>a</sup> (85)
GnRH + Ovsynch	32.1 <sup>a</sup> (81)	27.0 <sup>a</sup> (100)	23.0 <sup>a</sup> (100)
Luteal cyst			
Ovsynch	33.3 (9)	30.0 (10)	30.0 (10)
GnRH + Ovsynch	12.5 (8)	22.2 (9)	22.2 (9)
$< 1$ ng/mL plasma $P_4$			
Ovsynch	19.2 (52)	19.1 (68)	13.6 (66)
GnRH + Ovsynch	29.0 (62)	24.4 (78)	20.5 (78)
$> 1$ ng/mL plasma $P_4$			
Ovsynch	20.0 (25)	19.2 (26)	19.2 (26)
GnRH + Ovsynch	33.3 (24)	35.7 (28)	32.1 (28)

PR = pregnancy rate; Interaction Group  $\times$  Type (follicular or luteal) of cyst  $P < 0.05$ .

<sup>a</sup>  $P < 0.05$ .

### 3.6. Cows in anestrus

Pregnancy rates at days 30, 55 and 90 for cows in the Ovsynch (0/5; 1/8; 0/8) and GnRH + Ovsynch (1/8; 1/9; 1/9) groups were not evaluated statistically due to small sample size. Plasma P<sub>4</sub> concentration on day 0 for cows in the Ovsynch ( $0.25 \pm 0.10$  ng/mL) and GnRH + Ovsynch ( $0.55 \pm 0.29$  ng/mL) groups was not different. There was no difference in the proportion of cows with plasma P<sub>4</sub> concentration lower than 1 ng/mL in the Ovsynch (100%, 8/8) and GnRH + Ovsynch (75%, 6/8) groups. The positive predictive value for per rectum examination and ultrasonography of the genital tract to detect cows in anestrus was 87.5% (14/16).

## 4. Discussion

In a previous study, ultrasonography for pregnancy diagnosis was conducted at 27 d after AI, and the proportion of cows in diestrus was 46% [11]. Based on an expected interestrus interval of 17–24 d in cows not conceiving to the previous AI, ultrasonography was conducted at 30 d after AI in the present study to increase the number of cows with a CL at the time of ultrasonography. However, the proportion of cows in diestrus remained at 45%. Fifty-five percent of the cows in the present study were detected in metestrus, proestrus, with ovarian cysts and anestrus indicating that these cows had an estrous cycle of abnormal length probably due to various causes such as failure of previous synchronization, early regression of the CL, embryo loss with delayed return to estrus, or ovulatory failure of a return estrus. A treatment with GnRH at 7 d before non-pregnancy diagnosis on day 30 could be an alternative to reduce the number of cows without CL at the time a resynchronization program is initiated.

For cows in diestrus, there was a decrease in pregnancy rates for cows in the Quicksynch protocol compared to cows in the Ovsynch protocol. The lack of follicular wave synchronization reflected by the distribution of interestrus intervals in non-pregnant cows (Fig. 2) may explain this decreased pregnancy rate. In another study, there was no difference in pregnancy rate for cows subjected to Quicksynch and Heatsynch protocols for resynchronization after determination of non-pregnancy by ultrasonography [21]. In the present study, even though 78% of the cows in the Quicksynch group showed estrus activity  $\pm 1$  d of TAI, timing of AI may differ from studies where AIDE could have been more efficient [21]. Cows non-pregnant at ultrasonography on day 28 ( $\pm 1$ ) post AI subjected to a shortened Ovsynch protocol (PGF<sub>2 $\alpha$</sub>  day 0, GnRH on day 2 and TAI 16–20 h later) had a similar pregnancy rate to cows AIDE or TAI at 72–80 h after PGF<sub>2 $\alpha$</sub>  [10]. However, protocol responses were not compared to a protocol that synchronizes follicular waves [10]. Administration of GnRH at 7 d before a non-pregnancy diagnosis may be an alternative to synchronize the follicular wave prior to administration of PGF<sub>2 $\alpha$</sub>  at the non-pregnancy diagnosis and to insure the presence of a functional CL and a preovulatory follicle. Such a program would allow for a synchronized ovulation and permit a rapid TAI [22,23]. However, time of GnRH injection needs to be optimized based upon dynamics of interestrus intervals.

Because pregnancy rate for cows in the Quicksynch Group was acceptable when cows were AIDE using pedometers, the protocol was modified, and the Modified Quicksynch Group included AIDE using pedometers during the first four days and a TAI protocol (Ovsynch) assigned on day 4 for cows that had not expressed estrus. There was no difference in pregnancy rates for cows in the Modified Quicksynch and Ovsynch protocols. In the Modified Quicksynch protocol, a high proportion (72%) of cows was AIDE within 4 d after PGF<sub>2α</sub> administration. Therefore, this protocol may be an option to combine estrus detection and TAI to maintain pregnancy rates, and reduce the cost for resynchronization.

For cows in metestrus, there was a reduction in pregnancy rates for cows in the Heatsynch Group. In a previous study [11], the Heatsynch protocol increased pregnancy rates in cows in metestrus, since a high proportion (41%) of cows were AIDE after administration of ECP. In the present study, only 16% of the cows were AIDE after ECP, and this may explain the lower pregnancy rate. This difference between studies also reflects differences in efficacy of estrus detection as a management variable to consider when using the Heatsynch protocol. Cows in metestrus have already initiated a follicular wave and GnRH will not induce follicular turnover. Therefore, these cows may be more fertile if they are either AIDE following ECP in the Heatsynch protocol or delay the initiation of the Ovsynch protocol. The benefit of the GnRH + Ovsynch protocol probably relies on the delay of initiating the Ovsynch protocol, since the first dose of GnRH will not have any effect, but the Ovsynch is started 8 d later, at a more appropriate stage of the estrous cycle.

For cows in proestrus, there was no difference in pregnancy rates for cows in the Ovsynch or GnRH + Ovsynch groups. This is in agreement with a previous study where pregnancy rate was similar for the Ovsynch protocol administered in cows either in diestrus or proestrus [11].

In a previous study [24], the Ovsynch protocol was effective in cows with ovarian cysts. In the present study, pregnancy rates were higher for cows with ovarian cysts in the GnRH + Ovsynch protocol compared to cows in the Ovsynch protocol. Cows with ovarian cysts continue to have follicular waves [25,26]; therefore, the dominant follicle may or may not respond to GnRH treatment [27]. In the GnRH + Ovsynch protocol, cows received two doses of GnRH and may be more likely to have a CL at the time of PGF<sub>2α</sub>. In a previous study [28], however, pretreatment with GnRH at 7 d before the Ovsynch protocol did not increase pregnancy rate. It is possible that 7 d was not long enough for the recruited follicle to acquire ovulatory size and be responsive to the second dose of GnRH. However, an 8 d interval may enhance the ovulatory response such that a CL is present during the Ovsynch protocol coupled with recruitment of a new dominant follicle. The advantage of the GnRH + Ovsynch protocol in this study seems to be in cows with follicular cysts as determined by clinical diagnosis, but not based on P<sub>4</sub> concentrations.

Pregnancy losses between days 30 and 55 for cows in diestrus during the period from June to December were higher for cows in the Ovsynch protocol compared to cows in the Modified Quicksynch protocol. Higher pregnancy losses were reported for cows TAI using the Ovsynch protocol compared to cows AIDE after GnRH and PGF<sub>2α</sub> given 7 d apart during the summer [29]. In contrast, no differences in pregnancy losses were detected between cows AIDE after GnRH-PGF<sub>2α</sub> or TAI using the Ovsynch protocol [30] or AIDE after GnRH-PGF<sub>2α</sub> or TAI using the Heatsynch protocol [31].

Plasma P<sub>4</sub> concentrations have been used to differentiate luteal from follicular cysts [32,33] and in combination with ultrasonography gave an accurate classification of the

ovarian cyst [34,35]. In the present study, per rectum examination and ultrasonography of the genital tract were used to determine the stages of the estrous cycle, or presence of ovarian cysts or anestrus. The advantage of using both techniques in detection of ovarian cysts lies in the fact that ultrasonography allows for determination of luteinized structures [16,36] and per rectum palpation [19] to determine that the structure is in fact a follicle that never ovulated and not a cystic CL (i.e., CL has a line of demarcation and distortion in the shape of the ovary). In addition, since CL can still be observed at ultrasonography at 2 d after regression [37], per rectum palpation of the ovary will contribute to detecting a functional CL based on its morphology [11,19]. The positive predictive value for per rectum examination and ultrasonography of the genital tract was high for cows in diestrus, proestrus, and anestrus, but not as high for cows in metestrus. The specificity of per rectum examination and ultrasonography of the genital tract to detect cows with a luteal cyst was high (98.6%) but the sensitivity was low (31.5%). Farin et al. [16] reported high sensitivity (91.5%) and low specificity (70%) for ultrasonography to detect luteal cysts. The low sensitivity may be due to the cut-off value for plasma P<sub>4</sub> concentration being 0.5 ng/mL, and as a consequence the prevalence of luteal cysts was 70%. Comparing per rectum examination and ultrasonography of the genital tract, a high sensitivity and specificity were reported, and it was concluded that ultrasonography is effective for detecting luteal cysts [38]. Similarly, the present study indicated that when ultrasonography detects luteal cysts, plasma P<sub>4</sub> most likely will be greater than 1 ng/mL (positive predictive value of 90%).

In summary, cows in diestrus at the time of non-pregnancy diagnosis obtained acceptable pregnancy rates to the Ovsynch protocol, and in addition a Modified Quicksynch protocol may be an alternative to combine AIDE with TAI to reduce the time and cost needed for reinsemination. Cows in proestrus obtained an acceptable pregnancy rate to the Ovsynch protocol. For cows in metestrus, AIDE or delaying the Ovsynch protocol may be the alternative to increase pregnancy rate. For cows with ovarian cysts, administration of GnRH 8 d prior to the Ovsynch increased pregnancy rate.

Finally, the distribution of interestrus intervals and stages of the estrous cycle indicated that different strategies could be used to synchronize follicular growth before ultrasonographic diagnosis of non-pregnancy. Such strategies may increase the percentage of cows in diestrus that will respond to PGF<sub>2α</sub> and have a recently recruited dominant follicle for rapid re-insemination.

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