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Resynchronization of ovulation and timed insemination in lactating dairy cows III. Administration of GnRH 23 days post AI and ultrasonography for nonpregnancy diagnosis on day 30

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Abstract

The objective was to compare pregnancy rates to resynchronization and timed AI (TAI) protocols in lactating dairy cows that received GnRH at 23 d and were diagnosed not pregnant at 30 d after the pre-enrollment AI. Nonpregnant cows (624) at ultrasonography on day 30 (study day 0) were classified as diestrus (74.8%), metestrus (5.6%) and without a CL (19.5%). Cows in diestrus were assigned either to the GnRH group (PGF_{2α} on day 0, GnRH on day 2 and TAI 16 h later, $n = 238$) or the estradiol cypionate (ECP) group (PGF_{2α} on day 0, ECP on day 1, and TAI 36 h later, $n = 229$). Cows in metestrus were assigned to the Modified Heatsynch Group (GnRH on day 0, PGF_{2α} on day 7, ECP on day 8 and TAI on day 9, $n = 35$). Cows without a CL ($n = 122$) were classified either as proestrus (10.6%), ovarian cysts (7.5%) or anestrus (1.4%), and assigned to factorial treatments (i.e., use of GnRH versus CIDR) to either the GnRH group (GnRH on day 0, PGF_{2α} on day 7, GnRH on day 9 and TAI 16 h later, $n = 28$), the CIDR group (CIDR insert from days 0 to 7, PGF_{2α} on day 7, GnRH

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on day 9 and TAI 16 h later, $n = 34$), the GnRH + CIDR group (GnRH on day 0, CIDR insert from days 0 to 7, PGF_{2 α} on day 7, GnRH on day 9 and TAI 16 h later, $n = 32$), and the control group (PGF_{2 α} on day 7, GnRH on day 9 and TAI 16 h later, $n = 28$). For cows without a CL, plasma P₄ concentrations were determined on days 0, 7, 10 and 17 and ovarian structures determined on days 0, 7 and 17. Pregnancy rates were evaluated at 30, 55 and 90 d after the resynchronized AI. For cows in diestrus, there were no differences in pregnancy rates on days 30, 55 and 90 for cows in the GnRH (27.5, 26.5 and 24.2%) or ECP (29.1, 25.5 and 24.1%) groups. In addition, there were no differences in pregnancy losses between days 30 and 55 and 55 and 90 between the GnRH (7.0 and 8.6%) and ECP (9.8 and 5.4%) groups. For cows without a CL, GnRH on day 0 increased the proportion of cows with a CL on days 7 and 17 and plasma P₄ concentration on day 17 in cows with ovarian cysts but not for cows in proestrus. The CIDR insert increased pregnancy rate in cows with ovarian cysts but reduced pregnancy rate for cows in proestrus.

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1. Introduction

Systematic breeding programs including synchronization of ovulation and timed AI (TAI) enhance reproductive efficiency and consequently increase the profitability of dairy herds [1]. After first service, nonpregnant cows need to be detected as early as possible and then, resynchronized and inseminated in a timely manner to maintain high reproductive performance [2].

Different strategies for resynchronization of ovulation and TAI include synchronizing the follicular wave in advance by initiating the Ovsynch[®] protocol 7 d before nonpregnancy diagnosis [3–5]. An alternative was not to induce follicular wave synchronization after previous AI but administer PGF_{2 α} to induce luteolysis at the time of nonpregnancy diagnosis and then GnRH [2] or ECP [6,7] to induce ovulation for TAI.

Synchronization of ovulation for TAI is more effective when the first dose of GnRH in the Ovsynch protocol is able to induce ovarian follicular turnover [8,9]. Cows in proestrus obtained acceptable pregnancy rate to the Ovsynch protocol, and the distribution of interestrus intervals after prior AI indicated that most of the nonpregnant cows will be in the peri-estrous period around days 22–23 [7]. Therefore, administration of GnRH on days 22–23 after a previous AI should induce ovulation in the majority of the cows and result in formation of a CL and synchronization of an ovarian follicular wave.

In this study, GnRH was administered on day 23 after a pre-enrollment AI, and pregnancy diagnosis by ultrasonography was conducted on day 30. Nonpregnant cows were assigned to different protocols according to ovarian and uterine characteristics to resynchronize ovulation for TAI. The objective was to compare pregnancy rate in cows in diestrus after synchronization of ovulation with either GnRH or ECP and compare ovarian function and pregnancy rate in cows without a CL after synchronization of ovulation and TAI in a protocol including either GnRH or a progesterone (P₄) intra-vaginal insert.

2. Materials and methods

2.1. Study population

The study was conducted from January to March 2003 in a large commercial dairy in north central Florida with 3200 milking cows. The herd was divided into 14 lots according to levels of production and stage of lactation, and 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) three times daily. The TMR was formulated to meet or exceed requirements for lactation [10]. Cows were milked thrice daily and had a rolling herd average for milk production of 10,700 kg. Beginning at 60 d postpartum, cows received bST treatment (Posilac[®], 500 mg somatotribove zinc, subcutaneously; Monsanto, St. Louis, MO, USA) every 14 d during the entire lactation. Reproductive management consisted of a voluntary waiting period of 75 d that incorporated a Presynch–Ovsynch program [11] for first service and estrus detection using visual observation and a computerized pedometer system (Afimilk[®], S.A.E. Afikim, Kibbutz Afikim, Israel) for subsequent services. The study included 624 lactating dairy cows that received 100 µg i.m. of GnRH (2 mL of Cystorelin[®]; Merial Limited, Iselin, NJ, USA) 23 ± 1 d after AI and detected as nonpregnant at ultrasonography on day 30 ± 1 (study day 0). Cows with reproductive abnormalities (i.e., metritis, pyometra, uterine–ovarian adhesions) were not included in the study.

2.2. Study design

On study day 0, cows were classified into different stages of the estrous cycle or with ovarian cysts or in anestrus as determined by per rectum examination and ultrasonography of the genital tract [12] and assigned to different resynchronization protocols. On day 0, 74.8% (467/624) of the cows were in diestrus, 5.6% (35/624) in metestrus and 19.5% (122/624) without a CL (proestrus, ovarian cysts or anestrus).

Resynchronization protocols for cows in different stages of the estrous cycle or without a CL are described in Fig. 1.

Cows in diestrus ($n = 467$) were divided randomly into two experimental groups. Cows in GnRH group ($n = 238$) received 25 mg (i.m.) of PGF_{2α} (5 mL of Lutalyse[®] Sterile Solution, Pfizer Animal Health, New York, NY, USA) on day 0, 100 µg (i.m.) of GnRH on day 2 and TAI 16 h later. Cows in the ECP group ($n = 229$) received 25 mg (i.m.) of PGF_{2α} on day 0, 1 mg (i.m.) of ECP (0.5 mL of ECP[®] Sterile Solution, Pfizer Animal Health) on day 1 and TAI 36 h later. Cows were TAI 36 h instead of 48 h after ECP since AI at detected estrus was not performed.

Because of the small number of cows in metestrus, these cows were assigned to only one experimental group. Cows in metestrus ($n = 35$) received 100 µg i.m. of GnRH on day 0, 25 mg i.m. of PGF_{2α} on day 7, 1 mg i.m. of ECP on day 8 and TAI on day 9 (Modified Heatsynch Group). Cows were TAI at 24 h instead of 48 h after ECP, since cows in metestrus were expected to ovulate earlier.

On day 0, cows without a CL ($n = 122$) were classified as proestrus, with ovarian cysts or anestrus according to previously described criteria [12] and assigned randomly to four groups in a factorial arrangement. Cows in the GnRH group ($n = 28$) received 100 µg i.m.

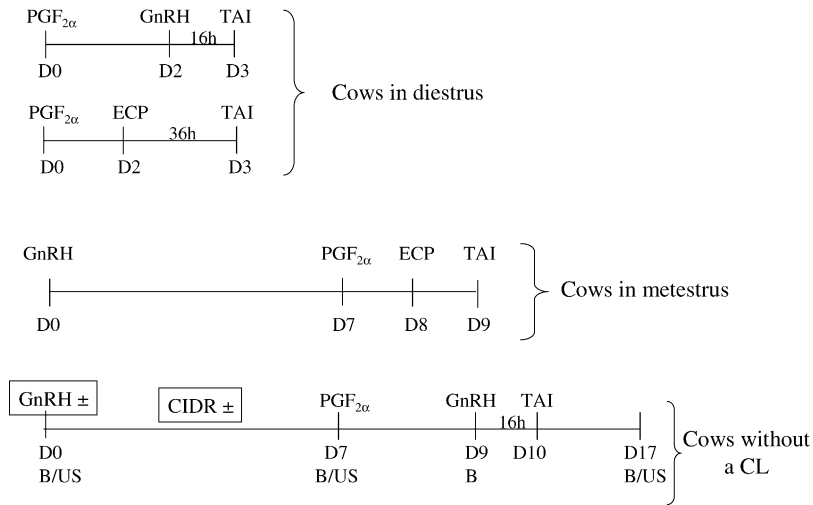


Fig. 1. Experimental design for cows in diestrus, metestrus or without a CL at the time of a nonpregnancy diagnosis (study day 0 = 30 d post-AI). B: blood sample; US: ultrasonography; TAI: timed insemination; ECP: estradiol cypionate.

of GnRH on day 0, 25 mg i.m. of PGF_{2α} on day 7, 100 μg i.m. of GnRH on day 9 and TAI 16 h later. Cows in the CIDR group ($n = 34$) received a CIDR insert (EAZI-BREED CIDR[®] insert, 1.38 g of progesterone, Pfizer Animal Health, New York, NY, USA) on day 0, 25 mg i.m. of PGF_{2α} and removal of the CIDR on day 7, 100 μg i.m. of GnRH on day 9, and TAI 16 h later. Cows in the GnRH + CIDR group ($n = 32$) received 100 μg i.m. of GnRH and a CIDR on day 0, 25 mg i.m. of PGF_{2α} and removal of the CIDR on day 7, 100 μg i.m. of GnRH on day 9 and TAI 16 h later. Cows in the control group ($n = 28$) received 25 mg i.m. of PGF_{2α} on day 7, 100 μg (i.m.) GnRH on day 9 and TAI 16 h later.

On day 0, parity, time of the year (season), days in milk (DIM), inseminator and housing lot were recorded for all the cows, and in addition, body condition score (BCS) was recorded for cows without CL.

2.3. Plasma P₄ concentrations

For cows without a CL, blood samples were collected on days 0, 7, 10 and 17 from the coccygeal vessels into evacuated tubes containing EDTA (Vacutainer[®], BD, Franklin Lakes, NJ, USA) and placed on ice immediately after collection. Blood samples on day 0 were obtained at the time of GnRH and CIDR administration, blood samples on day 7 at the time of PGF_{2α} administration and CIDR removal, blood samples on day 10 at the time of TAI and blood samples on day 17 at 7 d after TAI. Samples were centrifuged at 3000 × *g* for 30 min, and plasma stored at -20 °C until assayed for P₄ using a solid-phase, RIA (Coat-a-Count Progesterone, DPC[®]; Diagnostic Products Corporation, Los Angeles, CA, USA) with a standard curve, references and duplicates as previously described [7]. The sensitivity of the assay was 0.1 ng/mL. The intra-assay coefficients of variation for the high, medium and low duplicate samples were 5.7, 7.1 and 10.6%, respectively. Intra- and

inter-assay coefficients of variation for a luteal-phase reference sample (7.14 ± 0.69 ng/mL) were 9.6 and 10.4%, respectively.

2.4. Ovarian responses

For cows without a CL on day 0, per rectum examination and ultrasonography (Aloka 500 V with a linear-array, 5-MHz transrectal transducer, Aloka, Wallingford, CT, USA) of the genital tract were conducted on days 0, 7 and 17 to determine the presence of CL on the ovaries.

2.5. Pregnancy diagnosis to resynchronized AI

Pregnancy diagnosis by ultrasonography using a 5 MHz transrectal probe was carried out on day 30 ± 1 after the resynchronized AI. Cows were classified as: (1) pregnant: embryo proper with heartbeats and surrounded by a fluid-filled cavity representing the allantoic cavity as detected in the uterine lumen [13,14], (2) questionable pregnant: fluid in the uterine lumen and CL but no embryo observed or (3) not pregnant, cows that were AI at detected estrus before ultrasonography or nonpregnant at ultrasonography. On days 55 and 90, pregnancy was reconfirmed by per rectum examination of the genital tract based on previously described criteria [15]. Among cows evaluated at days 55 and 90 for pregnancy, the cows diagnosed nonpregnant at days 30 and 55 were not palpated subsequently at days 55 and 90, respectively. Cows classified as questionable pregnant were re-examined at day 55.

2.6. Statistical analysis

For cows in diestrus, baseline comparisons for parity (1, 2, 3+), DIM (quartiles), inseminator (A, B, C), and housing lot (A: lots with cooling system, B: first calf heifers in outside lots with shade and ponds, C: multiparous cows in outside lots with shade and ponds) were carried out to establish comparability among groups using the Chi-square test (PROC FREQ, SAS System). There was no difference in the distribution of cows between groups for different variables (parity, DIM, inseminator and housing lot) and no interactions between treatment group and these variables. Therefore, pregnancy rate on days 30, 55 and 90 and pregnancy losses between days 30 and 55, and days 55 and 90 for cows in diestrus were compared using the Chi-square test. Pregnancy rates for cows in metestrus were not statistically evaluated since they were assigned to one treatment due to the low number of cows in this category.

For cows without a CL (proestrus, ovarian cysts and anestrus), baseline comparisons for parity, DIM, inseminator, lot, body condition score (BCS $>$ or $<$ 3.0) and ovarian classifications (proestrus, ovarian cysts and anestrus) were carried out to establish comparability among groups using Chi-square test (PROC FREQ, SAS System). Since there was an interest in evaluating interactions between treatments and ovarian classification, categorical responses (CL on days 7 and 17, pregnancy rate on days 30, 55 and 90 and pregnancy losses between days 30 and 55 and days 55 and 90) were analyzed by multiple logistic regression using the backward elimination procedure in SAS (PROC

GENMOD, SAS system, [16]) including treatment, ovarian classification, parity, DIM, inseminator, housing lot, body condition score and interactions in the model. Rise in plasma P₄ concentrations from days 0 to 7 (ng/mL) and from days 10 to 17 (ng/mL) were analyzed using the repeated measures analysis of the mixed procedure of SAS using the autoregressive one covariance structure [17]. 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

3.1. Cows in diestrus

For the diestrus stage, 7.6% (18/238) of the cows in the GnRH group and 5.7% (13/229) of the cows in the ECP group were excluded from the study because they either had not received the complete protocols, or they were culled from the herd before pregnancy diagnosis on day 30. The distributions of cows by parity ($P = 0.42$), DIM ($P = 0.82$), inseminator ($P = 0.99$) and housing lot ($P = 0.81$) for the GnRH ($n = 220$) and the ECP ($n = 216$) groups were not different.

At day 30, in the GnRH group, 211 cows were diagnosed as either pregnant or not pregnant, five cows were diagnosed as questionable pregnant, and four cows were not subjected to ultrasonography. In the ECP group, 210 cows were diagnosed as either pregnant or not pregnant, one cow was diagnosed as a questionable pregnant, and five cows were not subjected to ultrasonography. One cow in the GnRH group that was pregnant at day 30 was culled from the herd before day 55.

Pregnancy rates on days 30, 55 and 90 and pregnancy losses between days 30 and 55 and days 55 and 90 for cows in diestrus subjected to either the GnRH or ECP groups were not different ($P > 0.15$; Table 1).

There was a group of cows ($n = 40$) that was not presented for early pregnancy diagnosis and enrollment on day 30 after a prior AI. These cows were either re-AI at 12 h after signs of estrus or palpated per rectum at 40 ± 5 d after AI and resynchronized using the Ovsynch protocol. The interval between previous AI and re-AI was 51.1 d for cows treated with GnRH on day 23 (35 cows) and 51.4 d for cows that did not receive the GnRH on day 23

Table 1
Pregnancy rates and pregnancy losses for cows in diestrus subjected to the GnRH or ECP groups.

Variable	Group	
	GnRH	ECP
	% (N)	% (N)
Pregnancy rate		
Day 30	27.5 (211)	29.1 (210)
Day 55	26.5 (219)	25.5 (216)
Day 90	24.2 (219)	24.1 (216)
Pregnancy losses		
Days 30–55	7.0 (57)	9.8 (61)
Days 55–90	8.6 (58)	5.4 (55)

(five cows). The interval between AI for cows in diestrus subjected to ultrasonography and rapid resynchronization was 33 d because they were TAI 3 d after a nonpregnancy diagnosis on day 30. Pregnancy rate was 22.5% (9/40) in cows not presented for ultrasonography at day 30 and 28.3% (119/421) for cows included either in the ECP or GnRH groups for resynchronization. The data from these 40 cows were not included in the statistical analysis since they were not enrolled in the study.

3.2. Cows in metestrus

For the metestrus stage, 22.8% (8/35) of the cows were excluded from the study since they either had not received the complete protocol or they were culled from the herd before pregnancy diagnosis on day 30. Pregnancy rate for cows in metestrus that received a Modified Heatsynch protocol was 18.5% (5/27) on day 30 and 14.8% (4/27) on days 55 and 90. Cows were supposed to be TAI at 24 h after ECP administration. However, five cows were TAI at 32 h after ECP, and three (60%) of them were pregnant.

3.3. Cows without a CL

For cows without a CL ($n = 122$), the distribution of cows for different variables (parity $P = 0.78$; DIM $P = 0.20$; inseminator $P = 0.27$; BCS $P = 0.31$; ovarian classification $P = 0.63$) other than housing lot ($P = 0.04$) were not different. For cows without a CL, 10.6% (66/624) were in proestrus, 7.5% (47/624) had ovarian cysts, and 1.4% (9/624) were anestrus. Cows in anestrus were not included in the statistical analysis due to the small number in this category.

For cows without a CL, 13.1% (16/122) were not evaluated on day 7 (five in the GnRH group, four in CIDR group, four in GnRH + CIDR group and three in control group), and 16.4% (20/122) were not evaluated on day 17 (six in GnRH group, five in CIDR group, seven in GnRH + CIDR group and two in control group). For pregnancy rate on day 30, 17.2% (21/122) of the cows were not evaluated because they were culled from the herd before pregnancy diagnosis (three in the GnRH group, six in the CIDR group, nine in the GnRH + CIDR group and three in control group), and 1.6% (2/122) of the cows were diagnosed as questionable pregnant (one in the GnRH group and one in the GnRH + CIDR group).

The number and proportion of cows with a CL on days 7 and 17 for cows in proestrus and with ovarian cysts for all four groups are described in Table 2. There was a tendency for an interaction between GnRH on day 0 and ovarian classification ($P < 0.11$) on the proportion of cows with a CL on day 7. GnRH administration on day 0 increased the proportion of cows with ovarian cysts that had a CL on day 7, but cows in proestrus had a high proportion of CL on day 7 regardless of whether or not they were treated with GnRH on day 0 ($P < 0.01$; Table 3). Cows with BCS greater than 3.0 were 3.6 times more likely to have a CL on day 7 than cows with BCS less than 3.0 ($P = 0.01$; Table 3). Cows with high plasma P_4 (> 1 ng/mL) on day 0 were six times more likely to have a CL on day 7 than cows with low plasma P_4 on day 0 ($P = 0.005$, Table 3). There was a tendency for an interaction between GnRH on day 0 and ovarian classification ($P < 0.12$) on the proportion of cows with a CL on day 17. GnRH administration on day 0 tended to increase the proportion of

Table 2

Ovarian responses on days 7 and 17 in cows classified as proestrus or with ovarian cysts for control, GnRH, CIDR and GnRH–CIDR groups

Group	Ovarian response	
	CL day 7	CL day 17
	% (N)	% (N)
GnRH		
Proestrus	75.0 (12)	72.7 (11)
Ovarian cysts	72.7 (11)	100 (11)
CIDR		
Proestrus	57.9 (19)	83.3 (18)
Ovarian cysts	45.4 (11)	72.7 (11)
GnRH + CIDR		
Proestrus	76.5 (17)	93.7 (16)
Ovarian cysts	81.8 (11)	88.9 (9)
Control		
Proestrus	78.6 (14)	85.7 (14)
Ovarian cysts	36.3 (11)	66.7 (12)

cows with a CL on day 17 in cows with ovarian cysts but not for cows in proestrus ($P = 0.06$; Table 4). Cows with BCS greater than 3.0 were 3.4 times more likely to have a CL on day 17 than cows with a BCS less than 3.0 ($P = 0.04$; Table 4). A CIDR insert from days 0 to 7 did not increase the proportion of cows with CL on day 7 and 17.

Plasma P_4 concentrations on days 0, 7, 10 and 17 for cows in proestrus or with ovarian cysts in all four groups are described in Table 5. Among cows with ovarian cysts, 43.5% had plasma P_4 less than 1 ng/mL (follicular cysts) and 56.5% had plasma P_4 greater than

Table 3

Number of cows, adjusted odds ratio (AOR), 95% confidence interval (CI) and levels of significance for the risk of presence of a CL on day 7 in cows classified as proestrus or with ovarian cysts and treated with GnRH on day 0 and adjusting for BCS and plasma P_4 concentration on day 0

Variable	CL on day 7			
	% (N)	AOR	95% CI	P value
Treatment*ovarian classification				
GnRH ovarian cysts	77.3 (22)	Referent	Referent	–
No GnRH ovarian cysts	40.9 (22)	0.1	0.02–0.5	0.005
GnRH proestrus	75.9 (29)	1.6	0.1–2.6	0.50
No GnRH proestrus	66.7 (33)	1.2	0.3–4.6	0.82
BCS				
<3.0	50.0 (24)	Referent	Referent	–
>3.0	69.5 (82)	3.6	1.2–10.5	0.01
P_4 on day 0				
<1 ng/mL	59.4 (69)	Referent	Referent	–
>1 ng/mL	78.4 (37)	6.2	1.7–22.4	0.005

Table 4

Number of cows, adjusted odds ratio (AOR), 95% confidence interval (CI) and levels of significance for the risk of presence of a CL on day 17 in cows classified as proestrus or with ovarian cysts and treated with GnRH on day 0 and adjusting for BCS

Variable	CL on day 17			
	% (N)	AOR	95% CI	P value
Treatment*ovarian classification				
GnRH ovarian cysts	95.0 (20)	Referent	Referent	–
No GnRH ovarian cysts	69.6 (23)	0.1	0.01–1.1	0.06
GnRH proestrus	85.2 (27)	0.2	0.02–2.4	0.22
No GnRH proestrus	84.4 (32)	0.2	0.02–2.2	0.19
BCS				
<3.0	70.8 (24)	Referent	Referent	–
>3.0	85.9 (78)	3.4	1.0–11.9	0.04

1 ng/mL (luteal cysts) on day 0. Progesterone concentrations were higher in cows diagnosed with ovarian cysts than cows in proestrus ($P < 0.01$). A CIDR*ovarian classification*day (i.e., days 0 and 7) interaction tended to be significant ($P < 0.09$) indicating that in proestrus cows with a CIDR progesterone concentrations at day 7 were lower compared to proestrus cows without a CIDR, whereas the CIDR did not have an adverse effect in cystic cows.

The increase of plasma P_4 concentration from days 10 to 17 was greater in cows with ovarian cysts that received GnRH on day 0, whereas there was no GnRH effect detected in proestrus cows (GnRH*ovarian classification*day, $P < 0.02$; Table 5).

Table 5

Least squares means \pm S.E.M. for plasma P_4 concentrations on days 0, 7, 10 and 17 in cows classified as proestrus or with ovarian cysts for control, GnRH, CIDR and GnRH–CIDR groups

Group	Ovarian response: Plasma P_4 concentration (ng/mL)			
	Day 0 ^a	Day 7 ^a	Day 10 ^b	Day 17 ^b
GnRH				
Proestrus	0.24 \pm 0.54	2.38 \pm 0.59	0.13 \pm 0.53	3.27 \pm 0.58
Ovarian cysts	1.82 \pm 0.57	3.02 \pm 0.54	0.61 \pm 0.50	4.34 \pm 0.47
CIDR				
Proestrus	0.59 \pm 0.57	1.73 \pm 0.72	0.14 \pm 0.58	3.54 \pm 0.71
Ovarian cysts	1.57 \pm 0.60	3.23 \pm 0.68	0.12 \pm 0.58	1.83 \pm 1.00
GnRH + CIDR				
Proestrus	0.43 \pm 0.60	2.55 \pm 0.89	1.00 \pm 0.71	2.12 \pm 0.71
Ovarian cysts	0.85 \pm 0.60	3.85 \pm 0.54	0.32 \pm 0.51	2.51 \pm 0.45
Control				
Proestrus	0.69 \pm 0.57	3.02 \pm 0.63	0.17 \pm 0.58	3.32 \pm 0.82
Ovarian cysts	1.41 \pm 0.54	2.50 \pm 0.56	1.0 \pm 0.50	1.59 \pm 0.71

^a Concentrations of P_4 from days 0 to 7: cystic vs. proestrus, $P < 0.01$; stage*CIDR*day, $P < 0.09$.

^b Concentrations of P_4 from days 10 to 17: stage*GnRH*day, $P < 0.02$.

Table 6

Pregnancy rates on day 30, 55 and 90 for cows classified in proestrus and with ovarian cysts for control, GnRH, CIDR and GnRH–CIDR groups

Group	Pregnancy rates		
	Day 30 % (N)	Day 55 % (N)	Day 90 % (N)
GnRH			
Proestrus	50.0 (12)	38.5 (13)	38.5 (13)
Ovarian cysts	16.7 (12)	8.3 (12)	8.3 (12)
CIDR			
Proestrus	5.9 (17)	5.9 (17)	5.9 (17)
Ovarian cysts	27.3 (11)	27.3 (11)	27.3 (11)
GnRH + CIDR			
Proestrus	21.4 (14)	20.0 (15)	20.0 (15)
Ovarian cysts	37.5 (8)	37.5 (8)	37.5 (8)
Control			
Proestrus	35.7 (14)	35.7 (14)	35.7 (14)
Ovarian cysts	27.3 (11)	27.3 (11)	18.2 (11)

Pregnancy rates on days 30, 55 and 90 for cows in proestrus and with ovarian cysts for all four groups are described in Table 6. GnRH on day 0 did not affect pregnancy rate on days 30, 55 and 90. There was an interaction between CIDR insert on day 0 and ovarian classification for pregnancy rates on days 30, 55 and 90 ($P < 0.05$). The CIDR insert on day 0 increased pregnancy rates on days 30, 55 and 90 in cows with ovarian cysts but decreased pregnancy rate for cows in proestrus (Tables 7–9). Cows with BCS greater than 3.0 tended to be 4.5 times more likely to get pregnant than cows with BCS less than 3.0 ($P < 0.10$; Tables 7–9).

Pregnancy rate on days 30, 55 and 90 for cows in anestrus were 25.0% (1/4) in the CIDR group, 0.0% (0/3) in the GnRH + CIDR group, 0.0% (0/2) in the control group, and there

Table 7

Number of cows, adjusted odds ratio (AOR), 95% confidence interval (CI) and levels of significance for the risk of pregnancy on day 30 in cows classified as proestrus or with ovarian cysts and treated with CIDR on day 0 and adjusting for BCS

Variable	Pregnancy rates on day 30			
	% (N)	AOR	95% CI	P value
Treatment*ovarian classification				
CIDR proestrus	12.9 (31)	Referent	Referent	–
No CIDR proestrus	42.3 (26)	4.9	1.3–19.3	0.02
CIDR ovarian cysts	31.6 (19)	4.0	0.9–18.0	0.06
No CIDR ovarian cysts	21.7 (23)	1.8	0.4–7.9	0.41
BCS				
<3.0	10.0 (20)	Referent	Referent	–
>3.0	30.3 (79)	4.5	0.9–22.2	0.06

Table 8

Number of cows, adjusted odds ratio (AOR), 95% confidence interval (CI) and levels of significance for the risk of pregnancy on day 55 in cows classified as proestrus or with ovarian cysts and treated with CIDR on day 0 and adjusting for BCS

Variable	PR on day 55			
	% (N)	AOR	95% CI	P value
Treatment*ovarian classification				
CIDR proestrus	12.5 (32)	Referent	Referent	–
No CIDR proestrus	37.0 (27)	4.6	1.2–19.3	0.02
CIDR ovarian cysts	31.6 (19)	4.0	0.9–18.0	0.06
No CIDR ovarian cysts	17.4 (23)	1.4	0.3–6.4	0.67
BCS				
<3.0	9.5 (21)	Referent	Referent	–
>3.0	37.5 (80)	4.3	0.9–21.1	0.07

PR: pregnancy rate.

Table 9

Number of cows, adjusted odds ratio (AOR), 95% confidence interval (CI) and levels of significance for the risk of pregnancy on day 90 in cows classified as proestrus or with ovarian cysts and treated with CIDR on day 0 and adjusting for BCS

Variable	PR on day 90			
	% (N)	AOR	95% CI	P value
Treatment*ovarian classification				
CIDR proestrus	12.5 (32)	Referent	Referent	–
No CIDR proestrus	37.0 (27)	4.6	1.2–19.3	0.02
CIDR ovarian cysts	31.6 (19)	4.0	0.9–18.0	0.06
No CIDR ovarian cysts	13.0 (23)	1.0	0.2–5.0	0.98
BCS				
<3.0	9.5 (21)	Referent	Referent	–
>3.0	26.3 (80)	4.1	0.8–20.3	0.08

PR: pregnancy rate.

were no cows in the GnRH group. Overall pregnancy rate for anestrus cows on days 30, 55 and 90 was 11.1% (1/9).

4. Discussion

Nonpregnant lactating dairy cows treated with GnRH on day 23 after AI and classified as diestrus at day 30 (75% of the cows) obtained similar pregnancy rates after inducing luteolysis with PGF_{2α} and resynchronizing ovulation for TAI with either GnRH or ECP. In addition, there was no difference in pregnancy losses between days 30 and 55 and days 55 and 90 between treatments for cows in diestrus.

Initiation of the Ovsynch protocol at 7 d before pregnancy diagnosis was used to resynchronize ovulation for TAI in lactating dairy cows [3–5]. Initiation of the Ovsynch protocol on day 20 after a previous AI for resynchronization of ovulation and TAI tended to

reduce pregnancy rate to first service in cows initiating bST injections at the time of the first GnRH of the Ovsynch protocol but not in control cows that initiated bST injections at 105 d in milk [3]. Pregnancy rates to second service were comparable in cows resynchronized (i.e., GnRH injection on day 20) or re-AI at detected estrus [3]. Initiation of the Ovsynch protocol on day 19 after previous AI did not affect pregnancy rate to first service, but resulted in a lower pregnancy rate to second service compared to Ovsynch initiated on days 26 or 33 [5]. However, administering GnRH at day 21 after AI did not reduce pregnancy rate to first service and resulted in a similar pregnancy rate to second service when compared to initiating the Ovsynch protocol on day 28 after a nonpregnancy diagnosis by ultrasonography [4]. In the present study, GnRH was administered in all cows on day 23 after AI, and control cows were not included since pregnancy rate was reduced after administering a shortened protocol (PGF_{2α} and ECP) in diestrus cows without GnRH injected 7 d earlier to synchronize the follicular wave [7].

The use of a shortened resynchronization protocol that did not attempt to synchronize follicle and CL development after a previous AI included the use of PGF_{2α} on day 28 to induce luteolysis in nonpregnant cows at ultrasonography, and synchronization of ovulation with either GnRH [2] or ECP [6,7]. Pregnancy rate for cows subjected to a shortened protocol (PGF_{2α}–GnRH) was similar to cows AI after PGF_{2α} combining estrus detection and TAI at 72–80 h [2], and similar between cows subjected to a shortened protocol (PGF_{2α}–ECP) compared to cows subjected to a Heatsynch protocol initiated on day 28 [6]. In contrast, pregnancy rate for cows subjected to a shortened protocol (PGF_{2α}–ECP) was reduced, compared to cows subjected to an Ovsynch protocol [7]. Protocols including ECP require efficient estrous detection and AI of cows at estrus before TAI at 48 h later [18] and may explain the difference between studies. Perhaps the success of shortened resynchronization protocols that did not consider synchronization of the follicular wave will depend on the efficiency and accuracy of estrus detection.

Early resynchronization and TAI of nonpregnant lactating dairy cows on days 27–29 after previous AI using PGF_{2α} alone or PGF_{2α} and GnRH resulted in a 22–23 d reduction in the calving to conception interval compared to nontreated cows [2]. In the present study, a control group of cows that did not receive GnRH on day 23 and ultrasonography on day 30 was not included, since the objective of the study was not to evaluate the advantage of early resynchronization using ultrasonography but the ovulatory protocols. However, in a group of concurrent cows that was not presented for early resynchronization, the interval between AI was 20 d longer compared to cows with a CL on day 30 and subjected to the two rapid resynchronization protocols.

In the present study, GnRH administered on day 23 increased the proportion of cows with CL (75%) compared to previous reports in the same herd (45 and 46%, [7,12]). Consequently, administration of PGF_{2α} induced luteolysis and permitted a synchronized ovulation in response to either GnRH or ECP for TAI. However, 25% of the cows still did not possess a mature CL at ultrasonography on day 30, and these cows had to be subjected to an alternative protocol to synchronize the follicular wave and induce formation of a CL. In addition to controlling follicular development and CL formation, GnRH treatment on day 23 may be inducing follicle turnover that would diminish a potential negative effect of a transitory pregnancy (i.e., cows experiencing embryo mortality) on follicular growth of the ovary ipsilateral to the previously pregnant uterine horn on day 30. Approaches to

maximize follicle turnover and CL formation at the time of GnRH treatment should optimize a resynchronized-TAI program. The inclusion of a CIDR insert between 14 and 21 d post AI may be useful to sustain follicular development, increase the response to GnRH treatment on day 23, and increase synchronization of follicle and CL development at the time of a nonpregnancy diagnosis on day 30. This approach may further optimize fertility at the TAI and warrants further investigation.

Pregnancy losses for cows subjected to synchronization of ovulation and TAI were 14.6% between days 32 and 74 [11]. In a previous resynchronization experiment [7], pregnancy losses between ultrasonography on day 30 and rectal palpation on day 55 for cows in diestrus and TAI were around 20%. In the present study, pregnancy losses between days 30 and 55 following the resynchronized service for cows in diestrus were 7.0 and 9.8% for GnRH and ECP groups, respectively. All resynchronized cows had received GnRH treatment at 23 d after the insemination. Thus, it was not possible to evaluate the effect of GnRH treatment at 23 d after insemination on pregnancy losses of cows that conceived to the resynchronized service.

Cows detected in metestrus (5.6%) were subjected to a Modified Heatsynch protocol. Pregnancy rate was high when cows in metestrus were AI at detected estrus on day 9 after the Heatsynch protocol [12]. In addition, pregnancy rate was low for cows in metestrus subjected to the Heatsynch protocol when a low proportion of cows were AI at detected estrus on day 9 [7]. In the present study, when the cows were TAI on day 9, pregnancy rate was lower than expected. Cows that were AI at 36 h after ECP (i.e., day 9.5) achieved a satisfactory pregnancy rate, but the number of cows was too small to compare 24 h versus 36 h or to compare the overall pregnancy rate of cows in metestrus with cows in diestrus. The use of GnRH to synchronize follicular wave emergence is not effective during metestrus [8,9]. Therefore, these cows probably need to be treated with PGF_{2α} 7 d later and then AI at detected estrus to optimize pregnancy rate. Alternatively, administration of a CIDR insert and estradiol could be utilized in metestrus cows to induce follicle turnover and synchronize follicular development for a subsequent TAI.

Cows in anestrus had a low overall pregnancy rate, but the number of cows in this category was too small to compare differences among groups. Since all cows received GnRH treatment on day 23 post AI, the proportion of cows with ovarian cysts was lower in the present study (7.5%), compared with previous studies (14.4 and 19.0%; [7,12]). In addition, the proportion of cows with luteal cysts (56.5%) was higher compared with a previous study (30.0%) in which GnRH was not given 7 d before diagnosis of nonpregnancy and diagnosis of the cystic condition [7].

In cows with ovarian cysts, GnRH on day 0 did not improve pregnancy rate. However, treatment with GnRH increased the proportion of cows with a CL on day 7, the rate of ovulation after the second GnRH of the Ovsynch protocol, and plasma P₄ concentration on day 17. The effect of GnRH treatment on cows with ovarian cysts has been controversial due to the lack of controls in many experiments and the proportion of cows recovering spontaneously [19,20]. Consequently, some studies reported beneficial [21–23] or no effect [24] of GnRH. Since cows with ovarian cysts also have follicular waves [20,25,26], the beneficial effect of GnRH is due to ovulation of a dominant follicle that may be present at the time of treatment and recruitment of a new potential ovulatory follicle rather than luteinization or regression of the cysts [22]. In the present study, GnRH on day 0 in cows

with ovarian cysts increased the proportion of corpora lutea on day 7, and this effect was not observed in cows at proestrus. Thus cows detected with multiple follicles, absence of a CL and lack of uterine tonicity (ovarian cysts) are less likely to ovulate spontaneously and form a CL compared with cows in proestrus with a dominant follicle and tonicity in the uterus.

Temporary P₄ exposure has been used previously to induce estrus, ovulation and CL formation in lactating dairy cows with ovarian cysts [27–31]. Progesterone treatment seems to restore the positive feedback effect of estradiol on GnRH release [32] that is compromised in cows with ovarian cysts [33–36]. However, the majority of these protocols were not intended to synchronize the follicular waves in order to synchronize ovulation and evaluate fertility to a TAI. Previous research indicated that induction of estrus in cows with ovarian cysts has to be combined either with high estrus detection [28,37] or TAI in order to increase pregnancy rate [38].

A treatment that combines GnRH on day 0, PGF_{2α} on day 7 and a CIDR insert from days 0 to 7 was used to induce follicular turnover, elevate P₄ during follicular growth and then induce an LH surge after CIDR removal and PGF_{2α} administration [28]. In the present study, a second treatment of GnRH (Ovsynch protocol) was used to induce ovulation. The benefit of the CIDR for 7 d before induction of luteolysis was to elevate P₄ concentrations, reduce the frequency of LH pulses and improve quality of a newly recruited dominant follicle for a subsequent synchronization of ovulation. Progesterone treatment reduced plasma LH concentrations and induced development of a new follicular wave in cows with follicular cysts [39] and cows in anestrus [40]. Therefore, it was expected that the CIDR insert would increase ovulation and pregnancy rate after CIDR removal. In the present study, the CIDR insert did not increase the proportion of CL or plasma P₄ concentration on day 17 but increased pregnancy rate in cows with ovarian cysts. In proestrus cows that received a CIDR, the lower concentration of progesterone at day 17 is due likely to a lack of CL development since the CIDR would have blocked ovulation of the proestrus follicle in this group. Subsequent insemination following removal of the CIDR insert would result in lower fertility associated with ovulation of a persistent follicle.

In summary, a GnRH treatment at 23 d after AI resulted in 75% of nonpregnant cows being in diestrus at 30 d after AI (day 0) and similar pregnancy rates were obtained using PGF_{2α} with either GnRH or ECP for resynchronization of ovulation and TAI. A different strategy for turning over the follicle may be necessary for cows in metestrus. A GnRH treatment on day 0 increased the proportion of CL at day 7 in cows with ovarian cysts, and the inclusion of a CIDR insert on day 0 increased pregnancy rate in cows with ovarian cysts but reduced pregnancy rate for cows in proestrus.

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References

- [1] Galligan DT. The economics of optimal health and productivity in the commercial dairy. *Rev Sci Technol* 1999;18:512–9.
- [2] Stevenson JS, Cartmill JA, Hensley BA, El-Zarkouny SZ. Conception rates of dairy cows following early not-pregnant diagnosis by ultrasonography and subsequent treatments with shortened Ovsynch protocol. *Theriogenology* 2003;60:475–83.
- [3] Moreira F, Risco CA, Pires MFA, Ambrose JD, Drost M, Thatcher WW. Use of bovine somatotropin in lactating dairy cows receiving timed artificial insemination. *J Dairy Sci* 2000;83:1237–47.
- [4] Chebel R, Santos JEP, Cerri RLA, Juchem S, Galvao KN, Thatcher WW. Effect of resynchronization with GnRH on day 21 after artificial insemination on pregnancy rate and pregnancy loss in lactating dairy cows. *Theriogenology* 2003;60:1389–99.
- [5] Fricke PM, Caraviello DZ, Weigel KA, Welle ML. Fertility of dairy cows after resynchronization of ovulation at three intervals following first timed insemination. *J Dairy Sci* 2003;86:3941–50.
- [6] Chebel RC, Cerri RLA, Galvao KN, Juchem SO, Santos JEP. Effect of rapid resynchronization of nonpregnant cows with estradiol cypionate (ECP) and PGF_{2α} on pregnancy rates (PR) and pregnancy losses (PL) in lactating dairy cows. *J Anim Sci* 2003;81(Suppl 1):182. abstract.
- [7] Bartolome JA, Sozzi A, McHale J, Melendez P, Arteche ACM, Silvestre FT, et al. 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. *Theriogenology* 2005;63:1628–42.
- [8] Vasconcelos JLM, Silcox RW, Rosa GJM, Pursley JR, Wiltbank MC. Synchronization rate, size of the ovulatory follicle and pregnancy rate after synchronization of ovulation beginning on different days of the estrous cycle in lactating dairy cows. *Theriogenology* 1999;52:1067–78.
- [9] Moreira F, de la Sota RL, Diaz T, Thatcher WW. Effect of day of the estrous cycle at the initiation of a timed artificial insemination protocol on reproductive responses in dairy heifers. *J Anim Sci* 2000;78:1568–76.
- [10] Nutrient requirements of dairy cattle (NRC). 7th revised ed. Washington DC: National Academy of Science; 2001.
- [11] Moreira F, Orlandi C, Risco CA, Lopes F, Mattos R, Thatcher WW. Effects of presynchronization and bovine somatotropin on pregnancy rates to a timed artificial insemination protocol in lactating dairy cows. *J Dairy Sci* 2001;84:1646–59.
- [12] Bartolome JA, Silvestre FT, Kamimura S, Arteche ACM, Melendez P, Kelbert D, et al. Resynchronization of ovulation and timed insemination in lactating dairy cows. I: use of the Ovsynch and Heatsynch protocols after nonpregnancy diagnosis by ultrasonography. *Theriogenology* 2005;63:1617–27.
- [13] Pierson RA, Ginther OJ. Ultrasonography of the bovine ovary. *Theriogenology* 1984;21:495–504.
- [14] Kastelic JP, Curran S, Pierson RA, Ginther OJ. Ultrasonic evaluation of the bovine conceptus. *Theriogenology* 1988;29:39–54.
- [15] Zemjanis R. Diagnostic therapeutic techniques in animal reproduction. Baltimore: Williams & Wilkins; 1962.
- [16] Agresti A. An introduction to categorical data analysis. 1st ed. New York: Wiley; 1996.
- [17] Littell RC, Henry PR, Ammerman CB. Statistical analysis of repeated measures data using SAS procedures. *J Anim Sci* 1998;76:1216–31.
- [18] Pancarci SM, Jordan ER, Risco CA, Schouten MJ, Lopes FL, Moreira F, et al. Use of estradiol cypionate in a pre-synchronized timed artificial insemination program for lactating dairy cattle. *J Dairy Sci* 2002;85:122–31.
- [19] Bierschwal CJ, Garverick HA, Martin CE, Youngquist RS, Cantley TC, Brown MD. Clinical response of dairy cows with ovarian cysts to GnRH. *J Anim Sci* 1975;41:1660–5.
- [20] Cook DL, Smith CA, Parfet JR, Youngquist RS, Brown EM, Garverick HA. Fate and turnover rate of ovarian follicular cysts in dairy cattle. *J Reprod Fertil* 1990;90:37–46.
- [21] Cantley TC, Garverick HA, Bierschwal CJ, Martin CE, Youngquist RS. Hormonal responses of dairy cows with ovarian cysts to GnRH. *J Anim Sci* 1975;41:1666–73.
- [22] Kesler DJ, Elmore RG, Brown EM, Garverick HA. Gonadotropin releasing hormone treatment of dairy cows with ovarian cysts I. Gross ovarian morphology and endocrinology. *Theriogenology* 1981;16:207–17.

- [23] Nanda AS, Ward WR, Williams PCW, Dobson H. Retrospective analysis of the efficacy of different hormone treatments of cystic ovarian disease. *Vet Rec* 1988;122:155–8.
- [24] Jou P, Buckrell BC, Liptrap RM, Summerlee AJ, Johnson W. Evaluation of the effect of GnRH on follicular ovarian cysts in dairy cows using trans-rectal ultrasonography. *Theriogenology* 1999;52:923–37.
- [25] Hamilton SA, Garverick HA, Keisler DH, Xu ZZ, Loos K, Youngquist RS, et al. Characterization of ovarian follicular cysts and associated endocrine profiles in dairy cows. *Biol Reprod* 1995;53:890–8.
- [26] Yoshioka K, Iwamura S, Kamomae H. Ultrasonic observations on the turnover of ovarian follicular cysts and associated changes of plasma LH, FSH, progesterone and estradiol-17b in cows. *Res Vet Sci* 1996;61:240–4.
- [27] Johnson AD, Ulberg LC. Influence of exogenous progesterone on follicular cysts in dairy cattle. *J Dairy Sci* 1967;50:758–61.
- [28] Archbald LF, Thatcher WW. Ovarian follicular dynamics and management of ovarian cysts. In: Van Horn HH, Wilcox CJ, editors. *Large Dairy Herd Management*. Champaign, IL: American Dairy Science Association; 1992. p. 199–208.
- [29] Douthwaite R, Dobson H. Comparison of different methods of diagnosis of cystic ovarian disease in cattle and an assessment of its treatment with a progesterone-releasing intra-vaginal device. *Vet Rec* 2000;147:355–9.
- [30] Wiltbank MC, Gumen A, Sartori R. Physiological classification of anovulatory conditions in cattle. *Theriogenology* 2002;57:21–52.
- [31] Zulu VC, Nakao T, Yamada K, Moriyoshi M, Nakada K, Sawamukai Y. Clinical responses of ovarian cysts in dairy cows after PRID treatment. *J Vet Med Sci* 2003;65:57–62.
- [32] Nanda AS, Ward WR, Dobson H. Lack of LH response to oestradiol treatment in cows with cystic ovarian disease and effect of progesterone treatment or manual rupture. *Res Vet Sci* 1991;51:180–4.
- [33] Dobson H, Alam MGS. Preliminary investigations into the endocrine systems of subfertile cattle: location of a common lesion (rate-limiting step). *J Endocrinol* 1987;113:167–71.
- [34] Refsal KR, Jarrin-Maldonado JH, Nachreiner RF. Basal and estradiol-induced release of gonadotropins in dairy cows with naturally occurring ovarian cysts. *Theriogenology* 1988;30:679–93.
- [35] De Silva M, Reeves JJ. Hypothalamic–pituitary function in chronically cystic and regularly cycling dairy cows. *Biol Reprod* 1988;38:264–9.
- [36] Kaneko H, Todoroki J, Noguchi J, Kikuchi K, Mizoshita K, Kubota C, et al. Perturbation of estradiol-feedback control of luteinizing hormone secretion by immunoneutralization induces development of follicular cysts in cattle. *Biol Reprod* 2002;67:1840–5.
- [37] Archbald LF, Norman SN, Tran T, Lyle S, Thomas PGA. Does GnRH work as well as GnRH and PGF_{2α} in the treatment of ovarian follicular cysts?. *Vet Med* 1991;86:1037–40.
- [38] Bartolome JA, Archbald LF, Morressey P, Hernandez J, Tran T, Kelbert D, et al. Comparison of synchronization of ovulation and induction of estrus as therapeutic strategies for bovine ovarian cysts in the dairy cow. *Theriogenology* 2000;53:815–25.
- [39] Calder MD, Salfen BE, Bao B, Youngquist RS, Garverick HA. Administration of progesterone to cows with ovarian follicular cysts results in a reduction in mean LH and LH pulses frequency and initiates ovulatory follicular growth. *J Anim Sci* 1999;77:3037–42.
- [40] Nation DP, Burke CR, Parton G, Steenson R, Macmillan KL. Hormonal and ovarian responses to a 5-day progesterone treatment in anoestrus dairy cows in the third week post-partum.. *Anim Reprod Sci* 2000;63:13–25.