Alternative programs to presynchronize estrous cycles in dairy cattle before a timed artificial insemination program¹

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ABSTRACT

The objective was to test potential presynchronization programs applied to cows before a timed artificial insemination (TAI) program to increase the percentage of cows ovulating in response to both GnRH injections of a TAI program and having a functional corpus luteum before the first GnRH injection of the TAI program. At calving, cows were blocked by lactation (1 vs. 2+) and assigned randomly to receive 1 of 5 presynchronization treatments. Two variants of the standard Presynch program were tested in which 2 injections of PGF_{2α} were administered 14 d apart with either 14 d (Pre14; n = 122), 12 d (Pre12; n = 123), or 10 d (Pre10; n = 151) intervening before a TAI program was initiated. Two other presynchronization programs consisted of administering a progesterone-releasing controlled internal drug release (CIDR) insert for 7 d plus PGF_{2α} administration at insert removal. Insert removal occurred either 10 d (CIDR10; n = 157) or 3 d (CIDR3; n = 117) before a TAI program was initiated. The TAI program was a standard Cosynch program [injection of GnRH 7 d before (GnRH-1) and 72 h after (GnRH-2) PGF_{2α} with TAI administered 72 h after PGF_{2α}]. Cosynch served as the control (n = 157), and cows were assumed to be starting this program at random stages of the estrous cycle. From a subset of cows per treatment (ranging from 49 to 51 cows each), blood samples were collected from coccygeal vessels by using evacuated tubes at d -28, -14, 0 (onset of TAI program), 7, 9, 14, and 21. Ovarian scans were conducted on d 0, 7, 9, 14, and 21 by transrectal ultrasonography. Diameters of follicles and corpus luteum were measured at each exam, and ovulation was determined on d 7 (response to GnRH-1 on d 0) and d 14 (response to GnRH-2 on d 10). Ovulatory incidence after GnRH-1 (47.1 to

67.3%) and GnRH-2 (78 to 90.2%) varied but did not differ among treatments. Before GnRH-1, progesterone concentrations were less in the CIDR3 treatment than in all other treatments. Before GnRH-2, progesterone was greater in the CIDR3 treatment than in all other treatments. Luteal regression and synchronization rate (successful luteolysis and ovulation after GnRH-2) did not differ among treatments. Pregnancy rate per AI at 32 and 60 d post TAI was less in CIDR3 cows than in cows in all other treatments. None of the Presynch treatments improved key responses (ovulation, luteolysis, and synchronization rate) known to improve fertility compared with a standard Cosynch program without presynchronization.

Key words: luteolysis, ovulation, timed AI

INTRODUCTION

When timed artificial insemination (TAI) programs were first attempted using Ovsynch [injection of GnRH 7 d before (GnRH-1) and 48 h (GnRH-2) after PGF_{2α} with TAI administered 16 to 20 h after GnRH-2], lactating dairy cows treated with this program between d 5 and 12 of the estrous cycle had a pregnancy rate per AI (PR/AI) advantage relative to cows treated at other stages of the cycle (Vasconcelos et al., 1999). On the basis of the hypothesis that fertility after a TAI program was related to the stage of the estrous cycle, presynchronization of estrous cycles was attempted before the Ovsynch program by using 2 injections of PGF_{2α} administered 14 d apart (Presynch). The second Presynch injection given 12 d before the onset of the TAI program (Moreira et al., 2001) resulted in a larger proportion of cows between d 5 and 12 of the estrous cycle at the onset of the TAI program. These cows had a significant improvement in PR/AI compared with cows at random stages of the estrous cycle (Moreira et al., 2001) and cows in subsequent experiments in which estrous cycles were presynchronized after administration of 1 (Cartmill et al., 2001) or 2 presynchronizing injections of PGF_{2α} (El-Zarkouny et al., 2004; Navanukraw

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et al., 2004; Galvão et al., 2007). In the latter 2 studies, the interval from the second Presynch injection to onset of the TAI program was 14 d rather than 12 d. In other experiments, however, single injections of PGF_{2α} given 3 d (Meyer et al., 2007), 10 d (LeBlanc and Leslie, 2003), or 12 d (Cartmill et al., 2001) before initiating a TAI program failed to improve PR/AI.

In subsequent experiments initiated after the present study, presynchronization of estrous cycles accomplished by injecting PGF_{2α} and GnRH (Bello et al., 2006) or by using an Ovsynch program before the TAI Ovsynch program (Souza et al., 2008) resulted in improved PR/AI. Applying an intravaginal progesterone insert (Chebel et al., 2006; Bicalho et al., 2007; Rutigliano et al., 2008) at various intervals before initiating a TAI program to appropriately stage the estrous cycle failed to improve PR/AI compared with a standard Presynch program but may have benefitted some anovulatory cows (Chebel et al., 2006) by inducing estrous cycles without affecting fertility (Cerri et al., 2009).

Improved PR/AI in TAI programs is associated with (1) greater ovulation response to the first GnRH injection of Ovsynch (Vasconcelos et al., 1999; Bello et al., 2006), (2) more cows having a functional corpus luteum (CL) at the first GnRH injection (Galvão and Santos, 2010), (3) greater ovulatory response to the first GnRH injection increasing ovulatory response to the second GnRH injection (Rutigliano et al., 2008), and (4) greater ovulation response after TAI (Galvão and Santos, 2010). Researchers concluded that presynchronization with 2 injections of PGF_{2α} (Presynch) and initiation of the Ovsynch 14 d later decreased ovulatory responses to the first and second GnRH injections. Therefore, a 14-d interval between presynchronization and the first GnRH injection might not be adequate to optimize fertility in dairy cows (Galvão and Santos, 2010).

The objective was to test potential presynchronization programs administered before a TAI program that might improve the percentage of cows having a functional CL, having elevated progesterone concentrations, and successfully ovulating in response to both GnRH injections of a TAI Ovsynch-like program. The hypothesis was that reducing the interval from the second Presynch PGF_{2α} injection from 14 to 10 d would increase the proportion of cows ovulating in response to GnRH-1. Further, applying progesterone via a controlled internal drug release (CIDR) insert for 7 d and removing the CIDR 10 d before GnRH-1 might have favorable effects on anovulatory or anestrous cows and enhance responses to a TAI program. In contrast, applying a CIDR for 7 d and removing it 3 d before initiation of a TAI program would produce unfavorable effects on responses to ovulation synchronization, including fertility.

MATERIALS AND METHODS

Experimental Cows

The current studies were approved by the Kansas State University Institutional Animal Care and Use Committee. Lactating Holstein cows were enrolled between October 2004 and January 2009 at the Kansas State University Dairy Teaching and Research Center. Cows were housed in covered freestalls and fed twice or thrice (summer) daily a TMR calculated to meet nutrient requirements for lactating dairy cows producing 50 kg of 3.5% milk (NRC, 2001). The diet consisted of alfalfa hay, corn silage, soybean meal, whole cotton seed, corn or milo grain, sweet bran, vitamins, and minerals. Cows were milked twice daily and treated with recombinant bST according to label directions.

Experimental Design

At calving, cows were blocked by lactation number (1 vs. 2+) and assigned randomly to receive 1 of 5 presynchronization treatments (Figure 1). Two permutations of the standard Presynch program were tested in which 2 injections of PGF_{2α} (5 mL of Lutalyse Sterile Solution; Pfizer Animal Health, New York, NY) were administered 14 d apart with either 14 d (**Pre14**; n = 122), 12 d (**Pre12**; n = 123), or 10 d (**Pre10**; n = 151) intervening before a TAI program was initiated. The rationale for decreasing the number of intervening days between the second PGF_{2α} injection and the onset of the TAI program was to place more cows at a time in their cycle when the first-wave dominant follicle would be likely to ovulate in response to GnRH-induced LH release (Vasconcelos et al., 1999; Moreira et al., 2000). Figure 1 shows the predicted days of the estrous cycle for these treatments. Two other presynchronization programs consisted of administering a progesterone-releasing Eazi-Breed CIDR cattle insert (Pfizer Animal Health) containing 1.38 g of progesterone for 7 d plus PGF_{2α} administration at insert removal. Insert removal occurred either 10 d (**CIDR10**; n = 157) or 3 d (**CIDR3**; n = 117) before a TAI program was initiated. The TAI program was a standard Cosynch program [100 µg of GnRH 7 d before (GnRH-1) and 72 h after (GnRH-2) 25 mg of PGF_{2α} with TAI administered 72 h after PGF_{2α}]. Cows administered Cosynch served as the control (n = 157), and cows were assumed to be starting this program at random stages of the estrous cycle. The CIDR3 treatment was a negative control; the hypothesis was that these cows would be at a disadvantage by starting the TAI program near estrus in a low-progesterone environment. Body condition scores were assigned on median d -28. The GnRH products

Figure 1. Five treatments designed to presynchronize the estrous cycle before the onset of a timed AI program (Cosynch; control) are illustrated. Injections of PGF_{20} were administered 14 d apart, with the second injection administered 14 d (Pre14), 12 d (Pre12), or 10 d (Pre10) before the onset of the Cosynch program. A controlled internal drug release (CIDR) insert containing 1.38 g of progesterone was inserted for 7 d and removed either 10 d (CIDR10) or 3 d (CIDR3; negative control) before the onset of the Cosynch program. Blood samples were collected and transrectal ovarian ultrasonography was performed in a subset of 49 to 51 cows per treatment as indicated. Probable days of the estrous cycle at the onset of the Cosynch program (random stages of estrous cycle) are illustrated. Abbreviations: B = blood samples; GnRH = 100 μg of gonadotropin-releasing hormone; PGF = 25 mg of PGF_{20} ; TAI = timed AI at 72 h after PGF_{20} ; US = ultrasonography. Color version available in the online PDF.

used were gonadorelin diacetate tetrahydrates (2 mL of Cystorelin, Merial Ltd., Duluth, GA; or 2 mL of OvaCyst, IVX Animal Health, St. Joseph, MO).

A new breeding cluster of cows was initiated every 2 wk at a median of 38 DIM, when cows were scored for body condition (Ferguson et al., 1994). Treatments had staggered starting times within cluster, so all treated cows within cluster were inseminated on the same day (Figure 1). Five technicians performed all AI, with 1 technician performing 84% of all AI. Multiple sires were used. Pregnancy diagnosis via transrectal ultrasonography (5.0 MHz linear-array transducer, Aloka 500V, Corometrics Medical Systems Inc., Wallingford, CT) occurred 32 and 60 d after TAI. A positive pregnancy outcome required the presence of anechoic uterine fluid and a large CL or anechoic uterine fluid and presence of a viable embryo.

To produce sufficient replication for assessing PR/AI [12 percentage point increase (El-Zarkouny et al., 2004) with $\alpha = 0.10$ and $\beta = 0.10$ for PR/AI ranging from 30

to 40% required 147 to 154 cows per treatment], more cows were assigned randomly to the 2 uniquely different treatments not previously tested in the literature (Pre10 and CIDR10) plus the Cosynch control. Therefore, numbers of cows per treatment for examining PR/AI were greater in the former 2 treatments and the Cosynch control (n ranged from 151 to 157) than in the latter 3 treatments (n ranged from 117 to 123).

Blood Sampling and Ultrasonography

From a subset of cows per treatment (ranging from 49 to 51 cows each), blood samples were collected from coccygeal vessels by using evacuated tubes at d -28, -14, 0 (onset of TAI program), 7, 9, 14, and 21 (Figure 1). Samples were stored on ice and transported to the laboratory for storage at 5°C until serum was harvested by centrifugation ($1,200 \times g$). Serum samples were stored at -15°C until they were assayed for progesterone concentration by radioimmunoassay (Skaggs et al.,

1986). Inter- and intraassay coefficients of variation for 8 assays were 10.9 and 8.2%, respectively, for a pooled serum sample that averaged 4.55 ± 0.11 ng/mL ($n = 41$). Concentrations of estradiol-17 β were measured by RIA (Perry et al., 1991) in blood serum collected on d 9 from the same subset of cows with some modifications. Blood serum before assay was extracted by 10 volumes of methyl T-butyl ether (HPLC grade, Fisher Scientific, St. Louis, MO). The radioligand (^{125} I-labeled 17 β -estradiol; 1,500 to 2,000 μ Ci/ μ g) was purchased from MP Biomedicals LLC (Solon, OH). Recovery of added mass (1, 2, 4, 8, and 16 pg) added in quadruplicate to 3 different bovine serum samples (300 μ L) averaged 109%. Parallelism was demonstrated by assaying 150-, 200-, 250-, and 300- μ L aliquots of bovine serum in quadruplicate and recovering 102% of that assayed in the 300- μ L sample of bovine serum. Inter- and intraassay coefficients of variation for 2 assays were 6.6 and 5.5%, respectively, for a pooled serum sample that averaged 6.33 ± 0.14 pg/mL ($n = 8$).

Measurements

On the basis of serum progesterone concentrations on d -28 and -14, cows were classified as anovulatory when progesterone was <1 ng/mL on both days; otherwise they were classified as having ovulated previously (cycling). Because cows in the CIDR10 treatment had been exposed to a CIDR in situ for 3 d on d -14, the concentration on d -14 must have exceeded 2 ng/mL for the cow to be classified as cycling when the concentration was <1 ng/mL on d -28. Stevenson et al. (2006) previously reported that in lactating dairy cows treated with a CIDR insert and having a functional CL at insert removal, the decrease in progesterone concentration was only 0.5 ± 0.1 ng/mL between the time of insert removal and a PGF $_{2\alpha}$ injection given 1 to 2 h later. These small differences in progesterone concentration indicated that the progesterone contribution of the CIDR insert to peripheral serum progesterone concentrations was small in the presence of a functional CL. Recently, Cerri et al. (2009) demonstrated that progesterone concentrations in the blood serum of lactating cows treated with a CIDR for 7 d beginning 2 d after PGF $_{2\alpha}$ -induced luteolysis were mostly subluteal (<1 ng/mL) after d 2 of administration.

Induced ovulation was defined to occur in previously anovulatory cows in response to treatment when progesterone exceeded 1 ng/mL on d 0 (onset of TAI program). The percentage of cows with elevated (≥ 1 ng/mL) progesterone on d 0, 7, 14, and 21 after initiation of Cosynch or those with reduced (<1 ng/mL) concentrations on d 9 (2 d post PGF $_{2\alpha}$) were calculated.

The percentage of cows with luteolysis was based on those with an observed CL and progesterone ≥ 1 ng/mL before PGF $_{2\alpha}$ injection and low progesterone concentrations 48 h later.

Ovarian scans were carried out on d 0, 7, 9, and 14 by transrectal ultrasonography (Figure 1). Diameter of follicles and CL (and their fluid-filled cavity) were measured at each exam. Ovulation response to GnRH-1 on d 0 and GnRH-2 on d 0 was determined on d 7 and 14, respectively, when a newly appearing CL was found at the site of a previously recorded follicle. Number of CL was counted at each exam, and their volume was calculated [$4/3 \times R^3 \times \pi$, where W = largest width and H = largest height of the structure; R = radius; $(W/2 + H/2)/2$, and $\pi = 3.14159$]. When a CL contained a fluid-filled cavity, the volume of the cavity was subtracted from the calculated CL volume. A successful synchronization occurred (synchronization rate) when luteolysis was achieved by 48 h after PGF $_{2\alpha}$ injection and a new CL was found 5 d after TAI at the same location as the largest follicle identified at 48 h after PGF $_{2\alpha}$.

Statistical Analyses

Various ovarian binomial characteristics and responses to hormonal injections (GnRH and PGF $_{2\alpha}$) were analyzed by logistic regression (procedure LOGISTIC, SAS Inst. Inc., Cary, NC). These included percentage of cows with elevated (≥ 1 ng/mL) or reduced (<1 ng/mL) progesterone concentrations, ovulation response to GnRH-1 and GnRH-2, and number of CL. The initial model included treatment ($n = 6$), lactation number (1 vs. 2+), season of year (hot = May to August; moderate = March, April, September, and October; cold = November to February), DIM at TAI (>76 or ≤ 76 d; median = 76), treatment $\times$ lactation number interaction, and BCS at the onset of the Pre14 treatment (>2.25 or ≤ 2.25 ; median = 2.25). The final model produced by backward stepwise selection of independent variables entered or retained in the model was based on a Wald statistic ($P < 0.10$).

Continuous variables and responses to hormonal injections (progesterone and estradiol concentrations, cavity-corrected CL volume, and follicle diameter) were analyzed by using a general linear model (procedure GLM, SAS Inst. Inc., Cary, NC). The basic model for determining volume of CL before each hormonal injection included treatment ($n = 6$), lactation number (1 vs. 2+), their interaction, BCS (>2.25 or ≤ 2.25 ; median = 2.25), and DIM (>76 or ≤ 76 ; median = 76 d). Models to analyze the diameter of the largest follicle before both GnRH injections included treatment ($n =$

6), cycling status (0 vs. 1), lactation number (1 vs. 2+), BCS (>2.25 or ≤ 2.25 ; median = 2.25), DIM (>76 or ≤ 76 ; median = 76 d), ovulation response (0 vs. 1), and the interaction of treatment and ovulation response. Simple Pearson correlation coefficients among CL characteristics and progesterone concentrations were determined by procedure CORR (SAS Inst. Inc.).

Pregnancy rates per AI at d 32 and 60 were analyzed as described previously for other binomial variables. The initial model included treatment ($n = 6$), cyclicity (0 vs. 1), lactation number (1 vs. 2+), season ($n = 3$), BCS (>2.25 or ≤ 2.25 ; median = 2.25), and DIM (>76 or ≤ 76 ; median = 76 d) at TAI. The final model produced by backward stepwise selection of independent variables entered or retained in the model based on a Wald statistic ($P < 0.10$) included treatment and cyclicity for both times of pregnancy diagnosis.

Relationships of various traits to the possible success of ovulation after both GnRH injections were modeled by using logistic regression (procedure LOGISTIC, SAS Inst. Inc.). All traits were regressed, and adjusted odds ratios were calculated. The final model produced by backward stepwise selection of independent variables entered or retained in the model was based on a Wald statistic ($P < 0.10$).

Estimates of pretreatment and posttreatment ovarian cyclicity assessed by serum progesterone concentrations described previously were analyzed by chi-squared frequency (procedure FREQ, SAS Inst. Inc.) by using the Cochran-Mantel-Haenszel statistics. Various comparisons in 34 anovulatory cows [reduced (<1 ng/mL) progesterone concentrations on d -28, -14, and 0 and no ultrasound-detected CL on d 0] were made in cows subsequently treated with a CIDR or not before Cosynch. The models included progesterone treatment (0 vs. 1) and lactation number (1 vs. 2+). Binomial and continuous variable models were applied as described previously for other like independent variables.

RESULTS

Ovarian Responses to GnRH-1

Responses to GnRH-1 are summarized in Table 1. As anticipated from the treatment design, progesterone concentrations were lesser ($P < 0.05$) in the CIDR3 treatment than in all other treatments. Progesterone concentrations were less ($P < 0.05$) in Pre10 cows than in Pre12 and Pre14 cows, which by experimental design, were likely earlier in their estrous cycles at the onset of the timed AI program (Figure 1). Cows with BCS ≤ 2.25 had lesser ($P < 0.05$) progesterone concentrations than did cows in greater (>2.25) body condition (2.5 ± 0.2 vs. 3.3 ± 0.3 ng/mL, respectively).

The proportion of cows with at least 1 CL at the time of GnRH-1 mirrored the percentages of cows with high progesterone with 1 exception (Table 1). Only 12% of CIDR3 cows had elevated (≥ 1 ng/mL) progesterone at GnRH-1, but 43% had at least 1 CL that was regressing (data not shown). Proportions of cows with progesterone ≥ 1 ng/mL at GnRH-1 that ovulated were 56.8, 68.6, 74.3, 65.8, 33.3, and 64.5% for Pre14, Pre12, Pre10, CIDR10, CIDR3, and Cosynch, respectively.

A greater ($P < 0.05$) proportion of cows with DIM greater than the median of 76 at TAI had elevated progesterone at GnRH-1 compared with cows with less than the median DIM at TAI (67.8 vs. 49.6%). The number of CL present at GnRH-1 and the total CL volume at the time of GnRH-1 were less ($P < 0.05$) in CIDR3 cows than in cows in all other treatments (Table 1). Mean diameters of the largest (putative dominant) follicle varied slightly among treatments (12.7 to 14.1 mm). Although ovulatory response to GnRH-1 looked numerically less in CIDR3 cows (43.1%), it did not differ ($P = 0.219$) from that in all other treatments ($>60\%$; Table 2).

Relationships of all traits to the success of ovulation after GnRH-1 were modeled. In the full model, only follicle diameter before GnRH-1 was associated ($P = 0.015$) with ovulation (Table 3). After backward stepwise selection, progesterone concentrations and largest follicle diameter were associated with ovulation after GnRH-1. Cows with follicle diameters >14 mm were 1.8 times more ($P = 0.019$; 95% CI = 1.10 to 2.89) likely to ovulate than cows with follicles ≤ 14 mm. Further, cows with progesterone concentrations ≥ 1 ng/mL were 1.7 times more ($P = 0.02$; 95% CI = 1.08 to 2.81) likely to ovulate than cows with progesterone concentrations <1 ng/mL before GnRH-1.

Ovarian Characteristics at Time of PGF_{2 α}

Various ovarian characteristics at the time of PGF_{2 α} injection are summarized in Table 1. Progesterone concentrations were greater ($P < 0.05$) in Pre14, Pre10, and CIDR10 cows than in Pre12, CIDR3, and Cosynch control cows. Cows in the CIDR3 treatment had fewer ($P < 0.05$) CL per cow than cows in all other treatments, and cows in the Cosynch control had an intermediate number of CL (Table 1). The volume of luteal tissue (CL) that formed after ovulation in response to GnRH-1 did not differ among treatments and was not affected by lactation number. In contrast, original CL volume before PGF_{2 α} injection and total CL volume (original CL plus new CL induced by GnRH-1) varied among treatments. Original CL volumes were greater ($P < 0.05$) in Pre14, Pre10, and CIDR10 treatments than in the Cosynch control (Table 1). Further, total

Table 1. Ovarian characteristics and serum progesterone concentrations at the time of or in response to the first (GnRH-1) and second (GnRH-2) GnRH injections and PGF_{2α} injection of a timed AI ovulation synchronization program (Cosynch)

Item	Treatment ¹						SE	P-value ²
	Pre14	Pre12	Pre10	CIDR10	CIDR3	Cosynch		
Cows, n	50	51	49	51	51	50		
GnRH-1								
Progesterone, ng/mL	3.9 ^a	3.7 ^a	2.4 ^b	3.0 ^{ab}	0.9 ^c	3.7 ^a	0.4	0.001
High progesterone, ³ %	74.0 ^a	68.6 ^a	71.4 ^a	74.5 ^a	11.8 ^b	62.0 ^a		0.001
Corpora lutea (CL), n	1.1 ^a	1.0 ^a	0.9 ^a	1.0 ^a	0.5 ^b	0.9 ^a	0.1	0.007
CL volume, cm ³	8.4 ^a	8.0 ^a	6.4 ^a	8.6 ^a	2.6 ^b	7.0 ^a	0.9	0.001
Follicle diameter, mm	13.5	14.1	13.5	12.7	12.7	13.4	0.6	0.524
PGF _{2α}								
Progesterone, ng/mL	5.9 ^a	4.3 ^b	5.5 ^a	5.8 ^a	3.4 ^b	3.7 ^b	0.4	0.001
High progesterone, ³ %	94.0 ^a	74.5 ^b	87.8 ^{ab}	92.2 ^{ac}	90.2 ^{ac}	74.0 ^b		0.006
Corpora lutea, n	1.9 ^a	1.6 ^{ac}	1.7 ^{ac}	1.7 ^a	1.2 ^b	1.5 ^{bc}	0.1	0.007
Orig. CL volume, cm ³	9.8 ^a	7.3 ^{bc}	9.5 ^{ab}	10.4 ^a	8.0 ^{abc}	6.3 ^c	0.9	0.008
New CL volume, cm ³	4.9	4.6	5.5	5.0	6.0	4.6	0.6	0.641
Total CL volume, cm ³	11.2 ^{ab}	9.1 ^{ac}	11.3 ^{ab}	12.4 ^b	8.4 ^c	8.4 ^c	0.8	0.002
GnRH-2								
Progesterone, ng/mL	1.0 ^a	0.5 ^b	0.7 ^{ab}	0.6 ^{ab}	1.5 ^c	0.9 ^a	0.1	0.001
Estradiol, pg/mL	1.6	1.8	1.7	1.5	2.0	1.7	0.2	0.659
Low progesterone, ⁴ %	82.0 ^{ac}	94.1 ^c	87.8 ^{ac}	84.3 ^{ac}	60.8 ^b	78.0 ^a		0.005
Follicle diameter, mm	13.8	14.3	13.9	14.0	15.1	13.8	0.4	0.021
Did not ovulate	12.3 ^{ab}	15.3 ^c	14.6 ^{bc}	11.2 ^a	15.6 ^c	14.0 ^{bc}	0.9	0.017 ⁵
Ovulated	14.0 ^{ab}	14.2 ^{ab}	13.8 ^a	14.5 ^a	15.1 ^b	13.8 ^a	0.4	0.017 ⁵

^{a-c}Means within a row with different superscripts differ ($P \leq 0.05$).

¹Injections of PGF_{2α} were administered 14 d apart, with the second injection administered 14 d (Pre14), 12 d (Pre12), or 10 d (Pre10) before the onset of the Cosynch program (see Figure 1). A progesterone-releasing controlled internal drug release (CIDR) insert was inserted for 7 d and removed either 10 d (CIDR10) or 3 d (CIDR3) before the onset of the Cosynch program.

²Treatment P -value.

³Percentage of cows with progesterone ≥ 1 ng/mL.

⁴Percentage of cows with progesterone < 1 ng/mL.

⁵Treatment $\times$ ovulation response interaction.

CL volume was greater ($P < 0.05$) in Pre14, Pre10, and CIDR10 treatments than in the CIDR3 treatment and Cosynch control. Volumes of the original CL (7.7 ± 0.6 vs. 9.4 ± 0.5 cm³) and total CL (9.4 ± 0.5 vs. 10.9 ± 0.5

cm³) were consistently less ($P < 0.05$) in first-lactation cows than in older cows. Progesterone concentration did not differ between first-lactation and older cows (4.96 ± 41 vs. 4.55 ± 0.28 ng/mL, respectively).

Table 2. Ovulation responses to the first and second GnRH injections, luteolysis, and synchronization rate of treated cows during the timed AI ovulation synchronization program (Cosynch) after various presynchronization treatments

Treatment ¹	n	Ovulation ²			
		GnRH-1, %	GnRH-2, %	Luteolysis, ³ %	Synchronization rate, ⁴ %
Pre14	50	62.0	86.0	93.9	86.0
Pre12	51	60.8	88.2	100	88.2
Pre10	49	65.3	79.6	95.7	79.6
CIDR10	51	64.7	86.3	100	86.3
CIDR3	51	43.1	90.2	83.0	86.3
Cosynch	50	62.0	78.0	93.0	76.0

¹Injections of PGF_{2α} were administered 14 d apart, with the second injection administered 14 d (Pre14), 12 d (Pre12), or 10 d (Pre10) before the onset of the Cosynch program (see Figure 1). A progesterone-releasing controlled internal drug release (CIDR) insert was inserted for 7 d and removed either 10 d (CIDR10) or 3 d (CIDR3) before the onset of the Cosynch program.

²Proportion of cows ovulating after the first or second GnRH injection of the Cosynch program.

³Proportion of cows having luteal regression (decrease in progesterone concentrations from ≥ 1 ng/mL before PGF_{2α} to < 1 ng/mL 48 h later).

⁴Synchronization rate (proportion of cows having luteal regression and ovulating after GnRH-2).

Table 3. Relationships of traits to ovulation success after the first GnRH injection (GnRH-1) of Cosynch

Trait	Level	n	Ovulation, %	P-value	AOR ¹	95% CI	
						Lower	Upper
Treatment ²	Pre14	50	62.0		1.12	0.49	2.56
	Pre12	51	60.8		1.17	0.51	2.71
	Pre10	49	65.3		0.93	0.40	2.16
	CIDR10	51	64.7		0.91	0.39	2.12
	CIDR3	51	43.1		1.91	0.81	4.53
	Cosynch	50	62.0	0.62			
Corpus luteum ³	Absent	89	53.9				
	Present	213	62.0	0.76	0.89	0.43	1.86
Progesterone, ³ ng/mL	<1	120	51.7				
	≥1	182	64.8	0.39	1.39	0.66	2.92
Follicle diameter, ³ mm	≤14	178	54.5				
	>14	123	67.5	0.02	1.88	1.13	3.11
BCS	≤2.25	194	59.8				
	>2.25	108	59.3	0.93	0.98	0.57	1.66
DIM	≤78	158	58.9				
	>78	144	60.4	0.74	0.92	0.56	1.51
Cyclicality ⁴	No	40	50.0				
	Yes	262	61.1	0.47	1.36	0.59	3.14
Lactation number	1	127	59.1				
	>1	175	60.0	0.72	1.13	0.59	2.16
Season	Cold	130	61.5		1.01	0.55	1.85
	Moderate	87	55.2		1.42	0.75	2.71
	Hot	85	61.2	0.44			
Summit milk, ⁵ kg	≤50	158	61.4				
	>50	144	57.6	0.43	0.78	0.42	1.45

¹Adjusted odds ratio.²Injections of PGF_{2α} were administered 14 d apart, with the second injection administered 14 d (Pre14), 12 d (Pre12), or 10 d (Pre10) before the onset of the Cosynch program (see Figure 1). A progesterone-releasing controlled internal drug release (CIDR) insert was inserted for 7 d and removed either 10 d (CIDR10) or 3 d (CIDR3) before the onset of the Cosynch program.³Assessed just before GnRH injection.⁴Cyclicality was based on serum progesterone concentrations 28, 14, and 0 d before the onset of a timed AI program.⁵Average of the largest 2 of the first 3 test-day milk weights after calving.

Ovarian Responses to GnRH-2

At GnRH-2, progesterone concentrations averaged <1 ng/mL in all treatments except CIDR3 (Table 1). Generally, more than 82% of cows had reduced (<1 ng/mL) progesterone by 48 h after PGF_{2α} compared with only 61% of CIDR3 and 78% of Cosynch control cows.

Diameters of the largest follicle differed ($P = 0.021$) among treatments, but an interaction of treatment and ovulation status also was detected (Table 1). Follicles that ovulated after GnRH-2, regardless of treatment, were more uniform in diameter, ranging from an average of 13.8 to 15.1 ± 0.4 mm. In contrast, average diameters of follicles that did not ovulate were more variable in size (11.2 to 15.6 ± 0.9 mm). In both scenarios, follicles with the largest diameter were identified in the CIDR3 treatment. Estradiol concentrations did not differ among treatments (Table 1); however, estradiol was greater ($P < 0.05$) in cows that ovulated after GnRH-2 (1.67 ± 0.2 vs. 1.28 ± 0.2 pg/mL) and in

cows that were cycling before the onset of the Cosynch program (1.74 ± 0.1 vs. 1.21 ± 0.3 pg/mL).

Ovulation response to GnRH-2 ranged from 79.6 to 90.2% but did not differ ($P = 0.357$) among treatments (Table 2) despite clear differences in progesterone concentrations 48 h after PGF_{2α} (Table 1). Luteal regression ranged from 83 to 100% but did not differ ($P = 0.37$) among treatments (Table 2). The CIDR3 cows, which started the Cosynch program in a low-progesterone environment, had the numerically lowest luteolytic success. Synchronization rate ranged from a low of 80% in Pre10 cows to a high of 88% in Pre12 cows, but did not differ ($P = 0.39$) among treatments (Table 2).

Relationships of all traits to the success of ovulation after GnRH-2 were modeled (Table 4). In the full model, only progesterone concentration at GnRH-2 was predictive ($P < 0.001$) of subsequent ovulation. After backward stepwise selection, the presence of CL at PGF_{2α} and progesterone concentrations before GnRH-2 was significantly associated with ovulation

Table 4. Relationships of traits to ovulation success after the second GnRH injection (GnRH-2) of Cosynch

Trait	Level	n	Ovulation, %	P-value	AOR ¹	95% CI	
						Lower	Upper
Treatment ²	Pre14	50	86.0		0.44	0.12	1.69
	Pre12	51	88.2		0.73	0.20	2.70
	Pre10	49	79.6		1.25	0.38	4.08
	CIDR10	51	86.3		0.93	0.27	3.17
	CIDR3	51	90.2		0.19	0.04	0.85
	Cosynch	50	78.0	0.22			
Corpus luteum at GnRH-1	Absent	89	78.7				
	Present	213	87.3	0.48	0.67	0.21	2.08
P4 ³ at GnRH-1, ng/mL	<1	120	80.8				
	≥1	182	87.4	0.40	2.03	0.68	6.08
Ovulation after GnRH-1	No	122	82.8				
	Yes	180	86.1	0.75	1.15	0.48	2.80
Corpus luteum at PGF _{2α}	Absent	25	64.0				
	Present	277	86.6	0.18	3.70	0.55	25.1
P4 at PGF _{2α} , ng/mL	<1	44	77.3				
	≥1	258	86.1	0.97	0.97	0.19	4.87
P4 at GnRH-2, ng/mL	<1	245	88.2				
	≥1	57	70.2	<0.001	5.99	2.32	15.5
Follicle diameter at GnRH-2, mm	≤14	136	86.8				
	>14	162	84.0	0.13	0.53	0.24	1.20
E2 ⁴ at GnRH-2, pg/mL	<1.56	128	81.3				
	≥1.56	130	87.7	0.64	0.83	0.37	1.86
BCS	≤2.25	194	84.0				
	>2.25	108	86.1	0.15	1.91	0.79	4.62
DIM	≤78	158	83.5				
	>78	144	86.1	0.96	1.02	0.47	2.21
Cyclicity ⁵	No	40	75.0				
	Yes	262	86.3	0.88	1.11	0.29	4.25
Lactation number	1	127	83.5				
	>1	175	85.7	0.08	2.56	0.89	7.38
Season	Cold	130	85.4		0.65	0.24	1.74
	Moderate	87	83.9		0.87	0.29	2.62
Summit milk, ⁶ kg	Hot	85	84.7	0.64			
	≤50	158	83.5				
	>50	144	86.1	0.40	0.65	0.24	1.76

¹Adjusted odds ratio.²Injections of PGF_{2α} were administered 14 d apart, with the second injection administered 14 d (Pre14), 12 d (Pre12), or 10 d (Pre10) before the onset of the Cosynch program (see Figure 1). A progesterone-releasing controlled internal drug release (CIDR) insert was inserted for 7 d and removed either 10 d (CIDR10) or 3 d (CIDR3) before the onset of the Cosynch program.³Progesterone.⁴Estradiol-17β.⁵Cyclicity was based on serum progesterone concentrations 28, 14, and 0 d before the onset of a timed AI program.⁶Average of the largest 2 of the first 3 test-day milk weights after calving.

success. Cows with at least 1 CL were 4.1 times more ($P = 0.007$; 95% CI = 1.47 to 11.3) likely to ovulate than cows with no CL at PGF_{2α}. Further, cows with progesterone concentrations <1 ng/mL were 3.6 times more ($P = 0.002$; 95% CI = 1.65 to 7.81) likely to ovulate than cows with progesterone ≥1 ng/mL before GnRH-2.

Progesterone and Post-AI Fertility

Progesterone concentrations at 4 d after TAI were greater ($P < 0.05$) in CIDR3 cows than in cows in all other treatments, but this difference was no longer detectable by d 11 post TAI (Figure 2). The greater

progesterone concentration in CIDR3 cows on d 4 was related to failure of the CL to regress in 8 of 47 cows. Although PR/AI in CIDR3 cows was consistently 50% of that in cows in other treatments at d 32 and 60 post-TAI (Figure 3), none of the presynchronization treatments improved PR/AI compared with the Cosynch control.

Correlations Between CL Traits and Progesterone

Simple correlations between the number of CL and progesterone concentrations at GnRH-1 ($r = 0.53$; $P < 0.001$; $n = 302$) were similar to those between total CL volume and progesterone concentrations at GnRH-1 (r

Figure 2. Serum progesterone concentrations 4 and 11 d after timed AI for lactating dairy cows treated with the Pre14, Pre12, Pre10, CIDR10, CIDR3, or Cosynch treatments. Injections of PGF_{2α} were administered 14 d apart, with the second injection administered 14 d (Pre14), 12 d (Pre12), or 10 d (Pre10) before the onset of the Cosynch program (see Figure 1). A progesterone-releasing controlled internal drug release (CIDR) insert was inserted for 7 d and removed either 10 d (CIDR10) or 3 d (CIDR3; negative control) before the onset of the Cosynch program. On d 4, mean progesterone concentrations (SEM = 0.24) differed ($P < 0.001$) between CIDR3 and all other treatments, but no differences (SEM = 0.38) among treatments were detected on d 8. Color version available in the online PDF.

= 0.57; $P < 0.001$; $n = 212$). In contrast, magnitude of the correlation between the number of CL and total CL volume was less ($r = 0.33$; $P < 0.001$; $n = 212$). Similar correlations were calculated for these traits at the time of the PGF_{2α} injection. The correlation between the number of CL and progesterone concentrations ($r = 0.53$; $P < 0.001$; $n = 302$) was similar to that between total CL volume and progesterone concentrations ($r = 0.51$; $P < 0.001$; $n = 274$). Again, the correlation between the number of CL and total CL volume was of lesser magnitude ($r = 0.42$; $P < 0.001$; $n = 274$), but numerically greater than that observed at GnRH-1.

Effect of CIDR Treatment on Anovulatory Cows

Pretreatment ovarian cyclicity ranged from 69 to 84% before treatments were applied (based on progesterone concentrations on d -28 and -14; Figure 4). After treatments were applied, the proportion of cows with at least 1 sample of elevated progesterone (≥ 1 ng/mL)

on d -28, -14, and 0 ranged from 80 to 93% and did not differ at the onset of the Cosynch program (d 0).

Of 34 anovulatory cows [reduced progesterone (< 1 ng/mL) on d -28, -14, and 0 and no visible CL at GnRH-1] treated with a CIDR or no CIDR, a few responses were ameliorated by progesterone treatment (Table 5). Ovulatory response to GnRH-2 was greater ($P = 0.019$) in cows previously treated with progesterone. Synchronization rate was improved ($P = 0.019$) by CIDR treatment, and progesterone concentration 11 d after TAI tended ($P = 0.075$) to be greater than that in anovulatory cows not treated with progesterone. For a limited number of cows, PR/AI at d 32 ($P = 0.075$) and d 60 ($P = 0.082$) tended to be greater in cows previously treated with a CIDR insert.

DISCUSSION

The study objective was to test 5 potential pre-synchronization programs administered before a TAI

Figure 3. Pregnancy rates (PR) per AI at 32 and 60 d after timed AI for cows treated with the Pre14, Pre12, Pre10, CIDR10, CIDR3, or Cosynch treatments. Injections of PGF_{2α} were administered 14 d apart, with the second injection administered 14 d (Pre14), 12 d (Pre12), or 10 d (Pre10) before the onset of the Cosynch program (see Figure 1). A progesterone-releasing controlled internal drug release (CIDR) insert was inserted for 7 d and removed either 10 d (CIDR10) or 3 d (CIDR3; negative control) before the onset of the Cosynch program. On d 32 and 60 after timed AI, PR/AI in the CIDR3 treatment differed ($P < 0.01$) from that in all other treatments. Color version available in the online PDF.

program that might improve the percentage of cows with characteristics known to improve subsequent PR/AI. Improved ovulation response to GnRH-1 (Bello et al., 2006; Stevenson et al., 2007) and GnRH-2 (Galvão and Santos, 2010) should result in greater PR/AI. To that end, multiple characteristics were examined in more than 300 cows to which 5 treatments were applied. On the basis of previous literature in which clear differences in PR/AI were observed (El-Zarkouny et al., 2004; Navanukraw et al., 2004; Galvão et al., 2007), it was anticipated that differences would be detected in ovarian characteristics for cows to which presynchronization treatments (Pre14, Pre12, and Pre10) were applied before Cosynch. Examination of progesterone concentrations revealed that in cows with at least 1 CL, CL volume, diameter of the largest follicle, and ovulation occurrence in response to GnRH-1 were not affected by treatment. Further, PR/AI was similar among treatments, and no trend toward expected differences was detected for cows treated with common Presynch treatments (e.g., Pre14 or Pre12) as reported earlier (El-Zarkouny et al., 2004; Navanukraw et al., 2004; Galvão et al., 2007).

Galvão and Santos (2010) recently reported that presynchronization with 2 injections of PGF_{2α} and

Figure 4. Pretreatment cyclicity was based on serum progesterone concentrations 28 and 14 d before the onset of a timed AI program (Cosynch). Posttreatment cyclicity was based on serum progesterone concentrations 28, 14, and 0 d before the onset of a timed AI program. Injections of PGF_{2α} were administered 14 d apart, with the second injection administered 14 d (Pre14), 12 d (Pre12), or 10 d (Pre10) before the onset of the Cosynch program (see Figure 1). A progesterone-releasing controlled internal drug release (CIDR) insert was inserted for 7 d and removed either 10 d (CIDR10) or 3 d (CIDR3; negative control) before the onset of the Cosynch program.

Table 5. Responses assessed during the Cosynch program in anovulatory cows previously treated with progesterone (P4)¹

Item	Progesterone treatment ²			No CIDR	<i>P</i> -value ³
	CIDR10	CIDR3	CIDR total		
Cows, n	5	9	14	20	
GnRH-1 ⁴					
Ovulation, %	40.0 (2/5)	33.3 (3/9)	35.7 (5/14)	50.0 (10/20)	0.416
P4, ng/mL	0.3 ± 0.09	0.3 ± 0.06	0.32 ± 0.05	0.3 ± 0.07	0.937
P4 at PGF _{2α} , ng/mL	2.6 ± 0.7	1.3 ± 0.4	2.1 ± 0.4	1.2 ± 0.4	0.142
GnRH-2 ⁴					
Ovulation, %	80.0 (4/5)	100 (9/9)	92.9 (13/14)	55.0 (11/20)	0.019
Progesterone, ng/mL	0.3 ± 0.1	0.5 ± 0.09	0.48 ± 0.07	0.3 ± 0.06	0.138
Luteolysis, ⁵ %	100 (3/3)	85.7 (6/7)	90.0 (9/10)	100 (12/12)	0.273
Synchronization rate, ⁶ %	80.0 (4/5)	100 (9/9)	92.9 (13/14)	55.0 (11/20)	0.019
P4, ng/mL					
4 d post-TAI	1.4 ± 0.5	1.6 ± 0.4	1.5 ± 0.4	0.8 ± 0.4	0.123
11 d post-TAI	4.5 ± 0.9	3.1 ± 0.4	3.6 ± 0.4	2.3 ± 0.4	0.075
PR/AI, %					
d 32	0 (0/5)	25.0 (2/8) ⁷	15.4 (2/13)	0 (0/20)	0.075
d 60	0 (0/5)	25.0 (2/8)	15.4 (2/13)	0 (0/19)	0.082

¹Cows having no corpus luteum on d 0 and low progesterone concentrations (<1 ng/mL) on d -28, -14, and 0 from the onset of the Cosynch program.

²Treatment with a progesterone-releasing controlled internal drug release (CIDR) insert for 7 d and removed either 10 or 3 d before the onset of the Cosynch program.

³Comparisons between no CIDR and CIDR total.

⁴First (GnRH-1) or second (GnRH-2) injections of GnRH during the Cosynch program.

⁵Proportion of cows having luteal regression (decrease in progesterone concentrations from ≥1 ng/mL before PGF_{2α} to <1 ng/mL 48 h later).

⁶Synchronization rate (proportion of cows having luteolysis and ovulating after GnRH-2).

⁷One cow was culled before pregnancy diagnosis on d 32.

initiation of Ovsynch 14 d later decreased ovulatory responses of dairy cows to both injections of GnRH compared with a standard Ovsynch control, which suggests the 14-d interval between presynchronization and GnRH-1 is not adequate to predispose better fertility in dairy cows. The same group (Galvão et al., 2007) reported improved PR/AI for cows treated with a Pre11 compared with a Pre14 presynchronization scheme. In that study, improvement in PR/AI between Pre11 and Pre14 treatments was associated with increased ovulation to GnRH-1 for Pre11 cows. When comparing Pre14 and Pre10 treatments, a similar trend was observed for an increase in PR/AI for Pre10 cows (Figure 3). This trend was consistent with our anticipation that a greater ovulatory response to GnRH-1 would occur in Pre10 cows because more first-wave follicles were likely present at the onset of Cosynch (calculated cycle d 5 to 10) than in Pre14 (calculated cycle d 9 to 13) cows. Ovulatory responses to GnRH-1 in Pre14, Pre12, and Pre10 cows in the present study were in the 60% range and agreed with those reported by Galvão et al. (2007) for Pre11 cows. But in that study, ovulatory response for Pre14 cows was only 45% and produced a significant difference in ovulation response to GnRH-1 between Pre11 and Pre14 cows.

It was observed that cows with elevated progesterone and follicles >14 mm at GnRH-1 had greater incidences of ovulation after GnRH-1 and that cows with at least 1 CL at the time of PGF_{2α} and low progesterone at GnRH-2 also had greater ovulation after GnRH-2. These findings are consistent with suggested mechanisms by which stage of estrous cycle or follicular maturation predispose improve fertility resulting from a TAI program. Improved PR/AI in TAI programs is associated with (1) greater ovulation response to the first GnRH injection of Ovsynch (Vasconcelos et al., 1999; Bello et al., 2006), (2) more cows having a functional CL at the first GnRH injection (Galvão and Santos, 2010), (3) greater ovulatory response to the first GnRH injection increasing ovulatory response to the second GnRH injection (Rutigliano et al., 2008), and (4) greater ovulation response after TAI (Galvão and Santos, 2010).

In the present study, responses measured for the CIDR10 treatment were similar to those for the other standard Presynch treatments. Four other studies have incorporated a CIDR into a standard Pre14 Presynch program, with 11 d (Bruno et al., 2009), 12 d (Cerri et al., 2009), 13 d (Chebel et al., 2006), or 14 d (Bicalho et al., 2007) between CIDR insert removal and PGF_{2α} injection and the onset of a TAI program. Fertility was

not improved in any of these studies, but ovulation response to GnRH-1 was increased in one study (Bruno et al., 2009). Further, the treatment with a CIDR insert reduced the ovulation response to GnRH-1 compared with a Pre13 Presynch program. One study (Rutigliano et al. 2008) duplicated the CIDR3 treatment, but no decrease was detected in PR/AI in this treatment when compared with a Pre12 treatment in that study. Because of its lack of profertility effects on PR/AI, it seems that applying a CIDR insert as a presynchronization tool before TAI is not warranted.

In contrast, the CIDR may have indirect effects on fertility because of progesterone's ability to induce estrous cycles. A greater proportion of anestrus cows receiving a CIDR insert during the last 7 d between PGF_{2α} injections in a Pre13 treatment became cyclic by d 62 compared with cows that did not receive CIDR insert (Chebel et al., 2006). Further, a new or used autoclaved CIDR incorporated into a Pre12 treatment resulted in more induced estrous cycles and prevented shortened estrous cycles after AI (Cerri et al., 2009). Results of the present study support the concept that use of progesterone during a presynchronization treatment induces estrous cycles in anovulatory cows. In the present study, more cows, albeit in limited numbers, receiving a CIDR insert ovulated in response to GnRH-2 and had a greater progesterone concentration after AI compared with cows that did not receive a CIDR. Although fertility was not improved in other studies with a presynchronizing CIDR treatment (Chebel et al., 2006; Bicalho et al., 2007; Bruno et al., 2009; Cerri et al., 2009), fertility was increased in cycling vs. anovulatory cows. Nonetheless, no anovulatory cows in the present study conceived after the TAI except those that received a CIDR insert.

Progesterone concentrations before GnRH-1 and PGF_{2α} were equally correlated ($r > 0.5$) with the total volume of luteal tissue, indicating that measures of luteal volume and luteal production of progesterone are well correlated. Positive correlations of progesterone concentration with ultrasound-derived luteal area were reported previously (Kastelic et al., 1990), but were of slightly greater magnitude ($r = 0.72$ to 0.76) than those in the present study.

CONCLUSIONS

Results of the present study fail to support the findings that absence of CL at GnRH-1 increased rate of single ovulations (Galvão and Santos, 2010). This may be partly explained by the large incidence (70.5%) of cows in the present study having a CL at GnRH-1. Cows in the present study with larger follicles and elevated progesterone were more likely to ovulate after GnRH-1,

whereas cows with at least 1 CL at PGF_{2α} and baseline progesterone before GnRH-2 had the best ovulation responses after GnRH-2. These results support the conclusion (Galvão and Santos, 2010) that strategies to increase the proportion of cows with a CL or high progesterone at GnRH-1 should be sought. Ovulation after GnRH-2, however, is much more critical to pregnancy outcome than the success of ovulation to GnRH-1. Under conditions of this 1-herd long-term study, no trends in PR/AI were noted except for the negative effect of applying progesterone for 7 d and removing the CIDR 3 d before initiating the TAI protocol.

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