

Effectiveness of administration of gonadotropin-releasing hormone at Days 11, 14 or 15 after anticipated ovulation for increasing fertility of lactating dairy cows and non-lactating heifers[☆]

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Abstract

One strategy for improving fertility in cattle is mid-cycle administration of GnRH to increase progesterone secretion and delay luteolysis. This strategy might be especially useful during hot weather because heat stress increases uterine prostaglandin release and reduces development of the elongating embryo. A series of experiments was conducted to test the efficacy of GnRH for increasing fertility. There was no effect of administration of 100 µg GnRH at Day 11 after anticipated ovulation on pregnancy rates in virgin heifers subjected to timed artificial insemination (TAI) during the summer. Similarly, there was no beneficial effect of administration of GnRH at Day 11 after anticipated ovulation on pregnancy rates of lactating cows subjected to TAI in summer and winter. Three experiments tested effects of injection of GnRH at Days 14 or 15 after anticipated ovulation on pregnancy rates of lactating cows. The first experiment used 477 lactating cows subjected to TAI. Cows receiving GnRH at Day 14 had higher pregnancy rates in both summer and winter than cows receiving vehicle (20.3 versus 12.7%, $P < 0.02$). When this experiment was repeated during summer with 137 cows, there was a negative effect of GnRH treatment at Day 14 on pregnancy rate. In the third experiment, lactating cows during summer were inseminated at detected estrus and cows were assigned to treatment with either GnRH or vehicle at Days 14 or 15 after insemination. Pregnancy rates were 25.6% (32/125) for cows receiving vehicle, 20.7% (19/92) for cows receiving GnRH at Day 14, and 20.3% (16/79) for cows receiving GnRH at Day 15. In conclusion, GnRH administration at Days 11–15 after anticipated ovulation or estrus did not consistently increase pregnancy rates in either cool or warm seasons.

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1. Introduction

One of the approaches proposed to improve fertility in cattle is administration of GnRH or GnRH analogues at Days 11–15 after estrus. Injection of GnRH at this time can decrease estrogen secretion [1,2], likely due to luteinization of the dominant follicle [1,3,4]. In some cases, extended estrous cycle length [5] and increased progesterone secretion also occurred [1,4,6,7]. Administration of GnRH or its analogues at Days 11–14 has

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improved fertility in nulliparous beef heifers [8] and lactating dairy cows [7,9–13]. In contrast to these positive results, there was no favorable effect of similar treatments of GnRH or GnRH analogues on pregnancy rates in other studies [4,6,14,15]. In a meta-analysis of published results, Peters et al. [16] concluded that the overall effect of GnRH administration between Days 11 and 14 after anticipated ovulation was positive but that results were not consistent among studies.

Perhaps GnRH treatment is more effective at increasing pregnancy rate per insemination during periods of heat stress than in cool weather because circulating concentrations of progesterone can be reduced in cows subjected to heat stress [17]. In addition, the anti-luteolytic process may be compromised because heat stress can decrease growth of the filamentous stage conceptus [18] and increase prostaglandin- $F_{2\alpha}$ secretion from the uterus [19]. Beneficial effects of GnRH treatment at Days 11–12 after insemination on fertility have been observed in lactating dairy cows during heat stress [7,13]. The purpose of the present series of experiments was to evaluate the effectiveness of GnRH treatment at Days 11, 14 or 15 after anticipated ovulation for improving fertility of lactating cows and heifers and determine whether the beneficial effect of GnRH was greater during summer than winter.

2. Materials and methods

2.1. Experiment 1—GnRH administration at Day 11 after anticipated ovulation in heifers subjected to timed artificial insemination during heat stress

The experiment was conducted at a commercial dairy located in Trenton, FL, USA (29°37'N 82°49'W) from July to September, 2003 using 149 Holstein heifers. Data on air temperatures and humidities for the time period of this experiment and other experiments are presented in Fig. 1. Data were recorded at a nearby weather station (Alachua, FL, USA; 29°75'N 82°42'W) and downloaded from the Florida Automated Weather Network (<http://fawn.ifas.ufl.edu>).

Heifers ranged in age from 13 to 23 mo (mean = 539 d, S.D. = 76) and ranged in weight from 316 to 448 kg (mean = 360 kg, S.D. = 32). Heifers were maintained on grass pasture with supplemental grass hay. Heifers were randomly allocated to one of four treatments in a 2 × 2 factorial design with main effects of timing of insemination (protocol A versus B) and treatment (vehicle versus GnRH). The experiment was replicated twice with 70–79 heifers per replicate. Heifers were subjected to timed artificial insemination

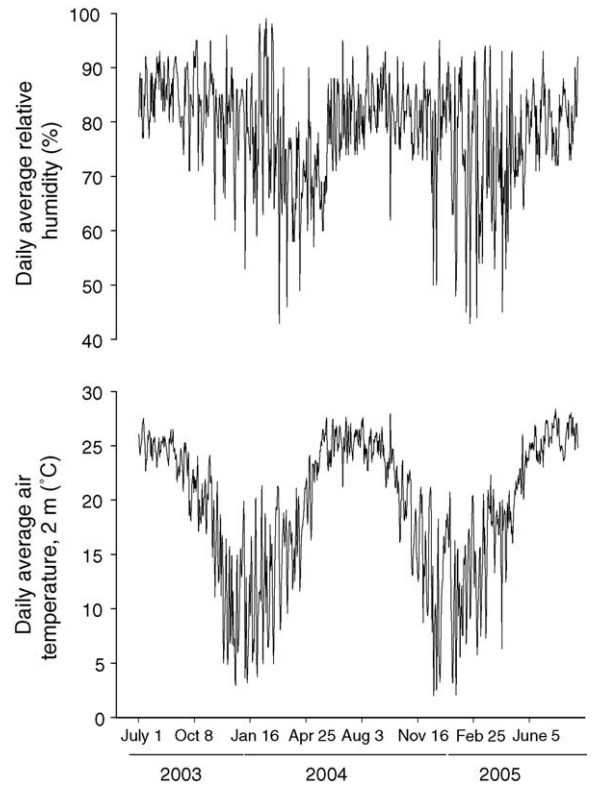


Fig. 1. Daily average air temperature (at 2-m elevation) and daily average relative humidity during the course of the experiments.

(TAI) based on a protocol published previously [20,21]. On Day –10 of the protocol (Day 0 = day of anticipated ovulation), heifers received 100 μ g (i.m.) of GnRH (gonadorelin diaacetate tetrahydrate; Fertagyl, Intervet Inc., Millsboro, DE, USA) and a new intravaginal progesterone-releasing device insert containing 1.38 g of progesterone (EAZI-BREED CIDR[®] insert, Pfizer Animal Health, New York, NY, USA). At Day –3, CIDR devices were removed and 25 mg (i.m.) of prostaglandin $F_{2\alpha}$ (PGF $_{2\alpha}$; Lutalyse, Pfizer Animal Health) was administered. A second GnRH treatment (100 μ g) was given 48 h after CIDR withdrawal (Day –1). Regardless of estrus behavior, heifers in protocol A were inseminated 24 h after the second GnRH injection (Day 0) and heifers in protocol B were inseminated at the same time as the second GnRH injection (Day –1). Two individuals conducted all inseminations and semen from one sire was used for all heifers. Heifers from each synchronization treatment protocol were randomly allocated to receive either 100 μ g of GnRH (i.m.) or an equivalent volume (2 mL of vehicle; 9 mg/mL of benzyl alcohol and 7.47 mg/mL of sodium chloride in water) at Day 11 after anticipated ovulation.

On the day of insemination and on Day 11 after anticipated ovulation, a 10-mL blood sample was collected via coccygeal or jugular venipuncture into heparinized tubes (Becton Dickinson, Franklin Lakes, NJ, USA) to measure the proportion of heifers successfully synchronized. An animal was considered synchronized if progesterone concentrations were <1 ng/mL on the day of insemination and >1 ng/mL on Day 11 after anticipated ovulation. A third blood sample was collected in a subset of 76 heifers at Day 15 after anticipated ovulation (i.e., 4 d after the injection of GnRH or vehicle) to determine the effect of GnRH treatment on serum concentrations of progesterone. Pregnancy was diagnosed by transrectal palpation at Days 44–51 after insemination.

Blood samples were stored on ice (~ 2 –4 h) until centrifugation at $2000 \times g$ for 20 min at 4°C to obtain plasma. Plasma was stored at -20°C until assayed for progesterone concentrations using a progesterone radioimmunoassay kit (Coat-a-Count[®]; Diagnostic Products Corp., Los Angeles, CA, USA). The sensitivity of the assay was 0.1 ng/mL and the intrassay and interassay CV were each 6%.

2.2. Experiment 2—GnRH administration at Day 11 after anticipated ovulation in lactating cows subjected to timed artificial insemination

This study took place at the University of Florida Dairy Research Unit (Hague, FL, USA; $29^\circ 46' \text{N}$ $82^\circ 25' \text{W}$). A total of 244 primiparous and multiparous lactating Holstein cows housed in freestall barns (equipped with a fan and sprinkler system) were used. Cows were fed a total mixed ration (TMR) to meet or exceed requirements recommended for lactating dairy cows, were milked three times a day, and received bovine somatotropin (Posilac, Monsanto Corp., St. Louis, MO, USA) according to manufacturer's recommendation. Cows were subjected to the OvSynch TAI program [22,23]; 100 μg i.m. of GnRH (Cystorelin; Merial Limited, Iselin, NJ, USA) was given at Day 0 of the protocol, 25 mg i.m. PGF_{2 α} (Lutalyse, Pfizer Animal Health) was given at Day 7, 100 μg i.m. of GnRH at Day 9, and cows were inseminated 16 h later (the day of anticipated ovulation). At the time of insemination (from January to September 2004), cows were between 76 and 594 d in milk (DIM; mean = 176, S.D. = 114). Multiple individuals conducted inseminations ($n = 7$) and multiple AI sires were used ($n = 45$).

Cows were randomly assigned within pairs to receive 100 μg i.m. of GnRH (Cystorelin) or an equivalent volume (2 mL) of vehicle (9 mg/mL benzyl alcohol and

7.47 mg/mL sodium chloride in water) at Day 11 after anticipated ovulation (i.e., 11 d after insemination). Rectal temperature was recorded in a subset of cows ($n = 134$) on the afternoon of Day 11 after TAI at 1500–1600 h. Pregnancy was diagnosed by transrectal palpation at \sim Day 46 after insemination.

2.3. Experiment 3—GnRH administration at Day 14 after anticipated ovulation in lactating cows subjected to timed artificial insemination

This study was conducted at two different locations using lactating Holsteins. Farm 1 was the University of Florida Dairy Research Unit at Hague, FL, USA, whereas Farm 2 was a commercial dairy in Chiefland, FL, USA ($29^\circ 30' \text{N}$ $82^\circ 52' \text{W}$). Cows from Farm 1 ($n = 306$) were inseminated from February to November 2004 and cows in Farm 2 ($n = 170$) were inseminated from June to October 2004. At both farms, primiparous and multiparous cows were used. At Farm 1, 307 cows were TAI between 76 and 590 DIM (mean = 187, S.D. = 102). Multiple individuals conducted inseminations ($n = 7$) and multiple AI sires were used ($n = 42$). At Farm 2, 171 cows were used for first service after calving using seven different sires and one inseminator. The TAI protocol was designed to achieve insemination at 60 ± 3 d in milk. Cows in both farms were housed in freestall barns equipped with fans and sprinklers, were fed a TMR, were milked three times a day, and received Posilac (Monsanto) according to manufacturer's directions.

Cows in Farm 1 were subjected to an OvSynch protocol as described for Experiment 2 with Cystorelin[®] (Merial). Cows for Farm 2 were subjected to a TAI protocol that incorporated a pre-synchronization with PGF_{2 α} [24] and the CIDR-Synch ovulation synchronization protocol [25]. Cows received two i.m. injections of 25 mg PGF_{2 α} (Lutalyse) 14 d apart, starting on Days 21–27 DIM. At 12 d after the second PGF_{2 α} injection, a timed ovulation synchronization protocol was initiated. Cows received 100 μg i.m. of GnRH (Cystorelin) and a new EAZI-BREED CIDR. Seven days later, CIDR inserts were removed and 25 mg i.m. of PGF_{2 α} was given. Cows received a second i.m. treatment of 100 μg of GnRH at 72 h after CIDR withdrawal. Estrus was detected using tail chalk or KaMar estrus detection patches (KAMAR Inc., Steamboat Springs, CO, USA). Cows observed in estrus at 24 or 48 h after CIDR removal were inseminated at estrus. Cows not observed in estrus were inseminated 72 h after CIDR withdrawal. Ovulation was anticipated to occur 72 h after CIDR withdrawal. All animals received the

GnRH injection at 72 h, regardless of estrus behavior. Cows were also randomly assigned within pair to receive either 100 µg i.m. of GnRH (Cystorelin) or vehicle (as for Experiment 2) at 14 d after anticipated ovulation. Pregnancy was diagnosed by transrectal palpation at ~Day 45 after insemination.

Rectal temperature was recorded in a subset of 100 cows in Farm 1 and 39 cows in Farm 2 at 1500 h of Day 14 after anticipated ovulation.

2.4. Experiment 4—GnRH administration at Day 14 after anticipated ovulation in lactating cows subjected to timed artificial insemination during heat stress

This study took place at the University of Florida Dairy Research Unit with inseminations in April–June 2005. Cows were housed in a freestall barn with fans and sprinklers, fed a total mixed ration (TMR), milked three times a day, and received bovine somatotropin (Posilac, Monsanto) according to manufacturer's recommendation. A total of 137 primiparous and multiparous lactating Holstein cows ranging in DIM from 78 to 566 d (mean = 185, S.D. = 110) were subjected to an OvSynch protocol as described for Experiment 2, except that the GnRH product was Cystorelin. Multiple individuals conducted inseminations ($n = 4$) and multiple AI sires were used ($n = 22$).

Cows were randomly assigned within pair to receive 100 µg i.m. of GnRH (Cystorelin) or an equivalent volume (2 mL) of vehicle (9 mg/mL benzyl alcohol and 7.47 mg/mL sodium chloride in water) at Day 14 after anticipated ovulation (i.e., 14 d after insemination). Pregnancy was diagnosed by transrectal palpation at ~Day 46 after insemination.

2.5. Experiment 5—GnRH administration at Days 14 and 15 after detected estrus in lactating cows

This study was conducted at a commercial dairy in Chiefland, FL, USA. Cows were housed in freestall barns equipped with fans and sprinklers, were fed a TMR, were milked three times a day, and received Posilac[®] (Monsanto) according to manufacturer's directions. A total of 296 primiparous and multiparous lactating Holstein cows inseminated at detected estrus were used. Cows were inseminated from April to August 2005. At the time of insemination, cows were between 51 and 235 DIM (mean = 122, S.D. = 40).

Estrous synchronization was not practiced, although some cows were given GnRH, PGF_{2α}, or both, as per veterinary instructions. Estrus was detected using tail

chalk or KaMar estrus detection patches. Estrus detection patches were visually monitored twice (morning and afternoon) daily by the inseminator. When cows were first diagnosed in estrus in the afternoon, insemination was performed the next morning. When estrus was first detected in the morning, cows were inseminated at that time. Cows were bred by one inseminator and 31 different sires were used. Every other day of the experiment, cows were selected to receive injections at Days 14 or 15 after insemination. Within each day, cows were randomly assigned within a pair to receive 100 µg i.m. of GnRH (Cystorelin) or an equivalent volume (2 mL) of vehicle (9 mg/mL benzyl alcohol and 7.47 mg/mL sodium chloride in water). Pregnancy was diagnosed by transrectal palpation at ~Day 45 after insemination.

2.6. Statistical analysis

Data on pregnancy rate were analyzed by logistic regression with the LOGISTIC and GENMOD procedures of SAS (SAS for Windows, Release 8.02; SAS Inst., Inc., Cary, NC, USA). For the LOGISTIC procedure, a backward stepwise logistic model was used. Variables were continuously removed from the model by the Wald statistic criterion if the significance was >0.20. The Wald χ^2 statistic was used to determine the significance of each main effect that remained in the reduced model. The adjusted odds ratio (AOR) estimates and the 95% Wald confidence intervals from logistic regression were obtained for each variable that remained in the final statistical model following the backward elimination. Data were also analyzed by PROC GENMOD and *P* values for significant treatment effects are reported from this analysis. The full mathematical model for Experiment 1 included main effects of inseminator, treatment, protocol, replicate, replicate × protocol, replicate × treatment, replicate × inseminator, protocol × treatment, protocol × inseminator, treatment × inseminator. The full mathematical model for Experiment 2 included the effects of season of insemination (January–March versus April–September), treatment, and season × treatment. For Experiment 3, the full mathematical model included the effects of farm, treatment, season of insemination (warm versus cool season; Farm 1 = October–March versus April–September; Farm 2 = June–September versus October–November), and season × treatment, season × farm, and treatment × farm. In addition, a subset of data composed of cows from Farm 2 only was analyzed where the additional factor of estrus detection (yes or no) was included in the model. For

Experiment 4, the full mathematical model included the effects of treatment, month of insemination, parity (1 versus others), sire, DIM at insemination class (<150 d versus >150 d), parity × treatment, DIM class × treatment and month × treatment. For Experiment 5, the full mathematical model included the effects of treatment, season of insemination (April and May versus June–August), parity (1 versus >1), number of services (1, 2 and >2), DIM at insemination class (<150 d versus >150 d) and interactions of main effects with treatment. Since interactions were not significant, data were reanalyzed with main effects only.

Data on rectal temperatures were analyzed by least-squares analysis of variance using the GLM procedure of SAS. The model included effects of season (Experiment 2) or season, farm and farm × season (Experiment 3).

A meta-analysis was performed using Mantel–Haenszel procedures available using software downloaded from <http://www.pitt.edu/~super1/lecture/lec1171/index.htm>. Three analyses were performed—using all experiments, experiments with GnRH treatment at Day 11, and experiments with GnRH treatment at Days 14 or 15.

3. Results

3.1. Experiment 1—GnRH administration at Day 11 after anticipated ovulation in heifers subjected to timed artificial insemination during heat stress

Based on progesterone concentrations measured at insemination and at Day 11 after anticipated ovulation, estrous cycles of 137/149 (92%) of the heifers were successfully synchronized. Pregnancy rate was not significantly affected by GnRH treatment or insemination protocol. This is true whether all heifers were considered (Table 1) or only those successfully synchronized (results not shown). There was also no significant effect of GnRH treatment at Day 11 on concentrations of plasma progesterone on Day 15. Values were 3.5 ± 0.19 ng/mL for heifers receiving vehicle and 3.6 ± 0.19 ng/mL for heifers receiving GnRH.

3.2. Experiment 2—GnRH administration at Day 11 after anticipated ovulation in lactating cows subjected to timed artificial insemination

Treatment with GnRH did not significantly affect pregnancy rate per insemination (Table 2); this was true for inseminations in both cool seasons (January–March)

Table 1

Descriptive statistics, adjusted odds ratio (AOR) estimates, and 95% Wald confidence intervals (CI) for effect of GnRH administration at Day 11 after anticipated ovulation and ovulation synchronization protocol on pregnancy rates of Holstein heifers during heat stress

	Pregnancy rate		AOR	95% Wald CI	P-value ^b
	Proportion ^a	%			
GnRH treatment ^c					
GnRH	20/78	25.6	1.29	0.59–2.83	0.41
Vehicle	14/71	19.7			
Protocol ^d					
B	20/79	25.3	1.34	0.61–2.95	0.41
A	14/70	20.0			

^a Data represent the number of females pregnant at Days 44–51 after insemination/total number of females inseminated.

^b Derived from PROC GENMOD.

^c Wald chi-square statistic = 0.54 (NS).

^d Wald chi-square statistic = 0.40 (NS).

and warm season (April–September) (results not shown). There was also no significant difference in pregnancy rate between seasons.

Rectal temperatures were higher ($P < 0.001$) for cows in the warm season (least-squares means \pm S.E.M.; 39.3 ± 0.07 °C) than for cows in the cool season (38.9 ± 0.07 °C).

3.3. Experiment 3—GnRH administration at Day 14 after anticipated ovulation in lactating cows subjected to timed artificial insemination

Injection of GnRH increased pregnancy rates at both farms (treatment, $P < 0.02$; treatment × farm,

Table 2

Descriptive statistics, adjusted odds ratio (AOR) estimates, and 95% Wald confidence intervals (CI) for effect of GnRH administration at Day 11 after anticipated ovulation and season of insemination on pregnancy rates of lactating Holstein cows subjected to timed artificial insemination

	Pregnancy rate		AOR	95% Wald CI	P-value ^b
	Proportion ^a	%			
GnRH treatment ^c					
GnRH	26/121	21.5	0.66	0.37–1.18	0.16
Vehicle	36/123	29.3			
Season ^d					
January–March	30/103	29.1	1.38	0.77–2.48	0.27
April–September	32/141	22.7			

^a Data represent the number of females pregnant at ~Day 45 after insemination/total number of females inseminated.

^b Derived from PROC GENMOD.

^c Wald chi-square statistic = 1.50 (NS).

^d Wald chi-square statistic = 1.38 (NS).

Table 3

Descriptive statistics, adjusted odds ratio (AOR) estimates, and 95% Wald confidence intervals (CI) for effect of GnRH administration at Day 14 after anticipated ovulation and season of insemination on pregnancy rates of lactating Holstein cows subjected to timed artificial insemination

	Pregnancy rate		AOR	95% Wald CI	P-value ^b
	Proportion ^a	%			
GnRH treatment ^c					
GnRH	49/241	20.3	1.76	1.07–2.89	0.02
Vehicle	30/236	12.7			
Season ^d					
October, November, February, March	40/187	21.4	1.76	1.08–2.87	0.02
May–September	39/290	13.5			

^a Data represent the number of females pregnant at ~Day 45 after insemination/total number of females inseminated.

^b Derived from PROC GENMOD.

^c Wald chi-square statistic = 4.94 ($P = 0.026$).

^d Wald chi-square statistic = 5.12 ($P = 0.024$).

non-significant; Table 3). Whereas pregnancy rates were lower in summer than winter ($P < 0.05$), the effect of GnRH was apparent in both seasons and the season \times treatment interaction was not significant.

Cows in Farm 2 were monitored for estrus. No cows were seen in estrus at 24 h after PGF_{2 α} , 4.7% (8/171) were detected in estrus at 48 h, 32.2% (55/171) at 72 h, and 63.1% (108/171) were not detected in estrus. Cows in estrus at 48 h were inseminated at that time, whereas other cows (those seen in estrus at 72 h and those not seen in estrus) were inseminated at 72 h. There was an estrus detection class (detected in estrus versus not detected) \times treatment interaction ($P < 0.03$) on pregnancy rate per insemination that occurred because GnRH was effective at increasing pregnancy rate for those cows displaying estrus [3/29 (10%) for control and 12/34 (35%) for GnRH] but was without effect for those cows not displaying estrus [7/54 (13%) for control and 4/54 (8%) for GnRH].

Rectal temperatures were higher ($P < 0.01$) for cows in the warm season (least-squares means \pm S.E.M., 39.4 ± 0.06 °C) than for cows in the cool season (39.1 ± 0.11 °C) and higher ($P < 0.001$) for Farm 2 (39.5 ± 0.10 °C) than for Farm 1 (39.1 ± 0.07 °C) but there was no farm \times season interaction.

3.4. Experiment 4—GnRH administration at Day 14 after anticipated ovulation in lactating cows subjected to timed artificial insemination during heat stress

Treatment with GnRH reduced pregnancy rate ($P < 0.05$; Table 4). Pregnancy rate was higher ($P < 0.02$) for cows inseminated at or before 150 DIM (30.3%, 20/66) than for cows inseminated after 150 DIM (12.7%, 9/71). There were no other significant main effects or interactions of GnRH treatment with other effects.

Table 4

Descriptive statistics, adjusted odds ratio (AOR) estimates, and 95% Wald confidence intervals (CI) for effect of GnRH administration at Day 14 after anticipated ovulation and Days in milk (<150 vs. >150 d) at insemination on pregnancy rates of lactating Holstein cows subjected to timed artificial insemination during heat stress

	Pregnancy rate		AOR	95% Wald CI	P-value ^b
	Proportion ^a	%			
GnRH treatment ^c					
GnRH	11/73	15.1	0.43	0.18–1.04	0.05
Vehicle	18/64	28.1			
Days in milk at insemination ^d					
<150 d	20/66	30.3	3.11	1.27–7.62	0.02
>150 d	9/71	12.7			

^a Data represent the number of females pregnant at ~Day 45 after insemination/total number of females inseminated.

^b Derived from PROC GENMOD.

^c Wald chi-square statistic = 3.55 ($P = 0.060$).

^d Wald chi-square statistic = 6.12 ($P = 0.013$).

3.5. Experiment 5—GnRH administration at Days 14 or 15 after detected estrus in lactating cows

Overall, pregnancy rate was higher ($P < 0.0001$) for cows inseminated in April and May (55/171, 32.2%) than for animals inseminated in June, July or August (12/125, 9.6%). There were, however, no other significant main effects or interactions of GnRH treatment with other effects. Pregnancy rates were 25.6% (32/125) for cows receiving vehicle at Days 14 or 15, 20.7% (19/92) for cows receiving GnRH at Day 14, and 20.3% (16/79) for cows receiving GnRH at Day 15.

3.6. Overall effectiveness of GnRH treatment as determined by meta-analysis

When data from multiple experiments were considered together by meta-analysis, there was no significant effect of GnRH on pregnancy rate. This was the case when all experiments were considered (odds ratio = 0.97; 95% CI = 0.63, 1.50), or whether experiments with GnRH treatment on Day 11 (odds ratio = 0.87; 95% CI = 0.50, 1.50) or Days 14 or 15 (odds ratio = 1.06; 95% CI = 0.68, 1.65) were considered separately.

4. Discussion

Overall, there was no significant effect of GnRH treatment on pregnancy rate. Treatment with GnRH at Day 11 after anticipated ovulation did not improve pregnancy rate of heifers or lactating cows in any experiment, whether animals were exposed to heat stress or not. Moreover, GnRH did not consistently improve fertility when given at Day 14 after anticipated ovulation or at Days 14 or 15 after insemination. In one experiment (Experiment 3), administration of GnRH at Day 14 after anticipated ovulation in cows subjected to TAI increased pregnancy rate of lactating cows in summer and winter at two locations. However, this positive effect could not be replicated either in lactating cows subjected to TAI or for cows inseminated at standing estrus. Indeed, GnRH reduced pregnancy rate in one of these studies.

The variability in response to GnRH is reminiscent of the results of the meta-analysis of published studies performed by Peters et al. [16] in which inconsistency among studies was noted. Variability in results could reflect either error in estimates of treatment effects because of small numbers of experimental units or variability in biological responses to GnRH. The number of animals used for the present studies varied

and could have been too small in some studies to detect significant differences or have lead to sampling errors that obscured the magnitude or direction of the treatment differences. However, meta-analysis of the entire data set, involving 1303 cows, indicated that there was no overall effect of GnRH.

Perhaps herds differ between each other or over time in the predominant biological response to GnRH treatment. Presumably, beneficial effects of GnRH post-insemination on fertility are related to its actions to cause LH release. Treatment with GnRH at Days 11–15 of the estrous cycle can decrease function of the dominant follicle [1–4] and increase progesterone secretion [1,4,6,7]. The reduction in estradiol-17 β secretion caused by GnRH should delay luteolysis and conceivably allow a slowly developing conceptus additional time to initiate secretion of interferon- τ . Low progesterone secretion may also compromise fertility in dairy cattle [26,27] and an increase in progesterone secretion caused by GnRH may facilitate embryonic development. Whether a herd responds to GnRH by undergoing follicular changes may depend upon the characteristics of follicular growth because a follicle must reach 10 mm in diameter to ovulate in response to LH [28]. Perhaps, herds that do not respond to GnRH with an increase in fertility are herds where many cows have lower follicular growth or follicular wave characteristics that do not result in sufficient follicular development at the time of injection.

One example of the potential importance of follicular dynamics in determining responses to GnRH is the expected response to GnRH treatment at Day 11 after anticipated ovulation. In the current studies, injection of GnRH at Day 11 after anticipated ovulation did not increase pregnancy rates in either lactating Holstein cows or nulliparous heifers. For lactating cows, there was the absence of an effect of GnRH at Day 11 in both summer and winter. This result, which agrees with other studies in which injection of GnRH at Day 11 does not affect fertility [6,14], is in contrast to other studies indicating that GnRH treatment at Day 11 can increase fertility of heifers [8] and lactating cows [7,11]. One factor that could influence the effectiveness of GnRH treatment at Day 11 is the number of follicular waves that an individual animal expresses. Animals with estrous cycles characterized by three follicular waves have a larger second-wave dominant follicle at Day 11 of the estrous cycle than animals with two-wave cycles [29–31] and thus the preponderance of cycle type (two-wave versus three-wave) within a herd may determine effectiveness of GnRH treatment at Day 11. There is variation from study to study in the relative frequency

of three-wave versus two-wave cycles, at least among Holstein heifers [29,32–34], and this variation is evidence for herd-to-herd variation in frequency of follicular wave patterns.

Even in animals with three-wave follicular cycles, Day 11 would appear to not be an optimal time of the estrous cycle for using GnRH to cause luteinization because the second-wave dominant follicle is smaller at Day 11 than at Days 14–15 in heifers [29,31] and lactating cows [30].

Results from a limited number of cows in Experiment 3 suggested that the effectiveness of GnRH at Day 14 after anticipated ovulation depends upon whether cows are detected in estrus. In that study, 37% of the cows were detected in estrus after the TAI program. Treatment with GnRH increased pregnancy rate in these cows but did not increase pregnancy rate in cows not detected in estrus. The proportion of cows detected in estrus within 3 d after GnRH is what would be expected for cows exposed to heat stress [35,36]. It is likely that GnRH did not affect pregnancy rate in the cows not detected in estrus, because this group included cows that were anovulatory at insemination or that were not successfully synchronized; GnRH would be unlikely to increase pregnancy rate in these animals.

It was hypothesized that beneficial effects of GnRH would be greater during heat stress because this condition can decrease growth of the filamentous stage conceptus [18], increase uterine prostaglandin $F_{2\alpha}$ secretion from the uterus [19] and reduce circulating concentrations of progesterone [17]. Beneficial effects of GnRH treatment at Days 11–12 after insemination on fertility have been observed in lactating dairy cows during heat stress [7,13]. There was no evidence, however, that GnRH was more effective during the summer. In particular, the increase in pregnancy rate caused by injection of GnRH at Day 14 during Experiment 3 was similar for cows inseminated in summer and winter. In other experiments conducted during the summer, GnRH was without beneficial effect.

In Experiment 1, there were no differences in pregnancy rates for Holstein heifers inseminated either at second GnRH injection (25.3%) or 24 h after GnRH (20.0%). This result was similar to results of Pursley et al. [23]; they reported little difference in pregnancy rates and no differences in calving rates between lactating cows inseminated at 0, 8, 16, or 24 h after the second GnRH injection of the OvSynch regimen. The pregnancy rates achieved with heifers in Experiment 1 were low compared to other studies in which heifers received a similar ovulation synchronization program [20,21]. The low fertility was not a result of delayed

puberty or unresponsiveness to the synchronization protocol because 92% of the heifers had both low progesterone concentrations during the expected peri-ovulatory period and high progesterone concentrations at the predicted luteal phase of the cycle. Perhaps some of these heifers classified as synchronized experienced short estrous cycles [22,37]. The experiment was conducted during the summer and it is also possible that heat stress reduced fertility. Although fertility in Holstein heifers does not always decline during the summer [38,39], there is one report [40] that heifers from a dairy farm in north-central Florida inseminated in summer were more than four times less likely to become pregnant to first insemination than heifers inseminated during the rest of the year. Perhaps the semen used (all from one sire) was of low fertility because the bull was infertile, the ejaculate was infertile, or the semen was mishandled during storage.

In conclusion, injection of GnRH at Days 11–15 after anticipated ovulation or insemination did not consistently increase pregnancy rates in heifers or lactating cows. That GnRH administration was effective in one study indicates that such a treatment may be useful for increasing pregnancy rate in some herds or situations. The practical benefits of GnRH treatment to increase fertility will remain elusive, however, unless predictive factors can be described that identify groups of cows most likely to benefit from GnRH treatment.

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