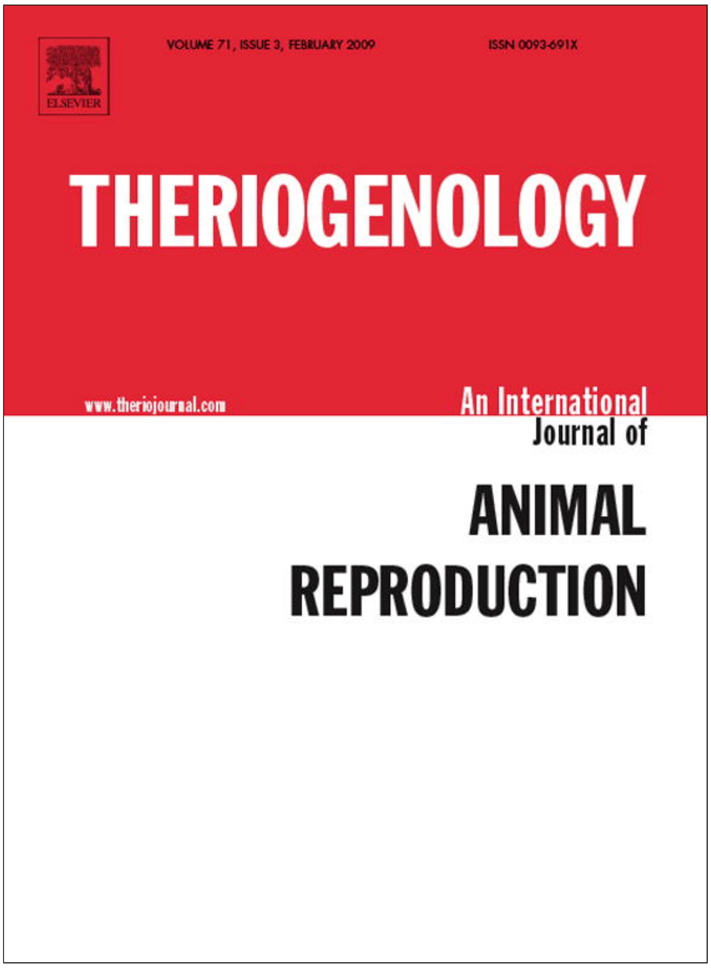
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Strategic treatment of anovular dairy cows with GnRH

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Abstract

The primary objective was to evaluate fertility of anovular dairy cows given GnRH 4 d after first postpartum timed AI (TAI). Secondary objectives were to determine ovulatory response to treatment, effect of treatment on serum progesterone (P_4) concentrations, and the proportion with a short luteal phase. Lactating Holstein cows (n = 1047) were submitted for first postpartum TAI using a Presynch + Ovsynch protocol. Anovular cows were identified from an initial 1047 lactating Holstein cows using transrectal ultrasonography, based on the absence of a CL at the first GnRH injection of a Presynch + Ovsynch protocol, and anovular cows were randomly assigned to receive either no further treatment (Control, n = 85), or 100 μg of GnRH 4 d after TAI (GnRH treated; n = 71). For GnRH treated cows, 51% responded by ovulating a follicle in response to GnRH treatment 4 d after TAI; however, pregnancies per AI (P/AI) did not differ between GnRH treated cows that ovulated (36%) compared to GnRH treated cows that did not ovulate (21%). There was a quadratic effect of P_4 at the $PGF_{2\alpha}$ injection of Ovsynch on P/AI, and cows with $P_4 \geq 1$ ng/mL at the $PGF_{2\alpha}$ injection of Ovsynch had greater P/AI (41%) than cows with $P_4 < 1$ ng/mL (12%); however, no treatment difference was detected. Overall, P/AI did not differ between control (30.1%) and GnRH treated (29.6%) treatments for synchronized cows. Although treatment of anovular cows with GnRH 4 d after TAI failed to improve fertility, variation among cows in serum P_4 at the $PGF_{2\alpha}$ injection of Ovsynch dramatically affected fertility of anovular dairy cows.

Keywords: Anovulation; Dairy cow; GnRH; Ovsynch; Fertility

1. Introduction

The first step to establishing pregnancy among a group of postpartum lactating dairy cows is to submit all cows for AI service in a timely manner. The adoption of programs that synchronize ovulation for timed AI (TAI), such as Ovsynch (synchronization regimen using sequential injections of GnRH and $PGF_{2\alpha}$ to precisely time ovulation for TAI [1] or Presynch (postpartum regimen using two injections of $PGF_{2\alpha}$ to synchronize estrous cycles before applying Ovsynch [2,3]) + Ovsynch, allow dairy producers to submit cows for AI without estrus detection. Thus, producers are not only able to designate a voluntary waiting period but also a maximum number of d postpartum at which all cows receive TAI within a herd.

Farms aggressively using Ovsynch or Presynch + - Ovsynch to submit all cows for first postpartum TAI are able to designate all cows calving within a given calendar week to receive TAI at a desired day postpartum, within a range of ±3 d. Because this system allows all cows to be submitted for TAI (regardless of estrus detection), cyclicity status of individual cows before TAI is unknown. One concern with using Ovsynch in this manner is that noncycling

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cows produce fewer pregnancies per AI (P/AI) and undergo more pregnancy loss than cycling herdmates [2,4,5]. The proportion of anovular cows submitted for first postpartum TAI is frequently reported to be between 20 and 30% [6–8], but ranges from 8% [9] to 55.7% [10].

A previous report showed that GnRH treatment 5 d after the GnRH + TAI of Presynch + Ovsynch tended to improve fertility [11]. Furthermore, the tendency for improved fertility was due to a treatment by cyclicity interaction, in which anovular cows treated with GnRH 5 d after TAI tended to have greater P/AI than untreated anovular cows. In that experiment, however, all anovular cows received a controlled drug releasing insert (CIDR) containing 1.38 g of progesterone (P_4) at the first GnRH injection of Presynch + Ovsynch for 7 d. Thus, it is unknown whether GnRH administration after TAI can improve P/AI in anovular cows not previously exposed to CIDR treatment, or if there is a synergistic interaction between P_4 before TAI and GnRH treatment after TAI.

The primary objective of this study was to evaluate P/AI for anovular dairy cows treated with GnRH 4 d after first postpartum TAI. Secondary objectives were to determine ovulatory response to GnRH treatment 4 d after TAI and the effect of GnRH treatment on circulating P_4, and proportion of anovular cows with a short luteal phase. Our hypothesis was that treatment of anovular cows with GnRH 4 d after first postpartum TAI would improve P/AI, possibly by reducing the proportion of cows undergoing a short luteal phase.

2. Materials and methods

2.1. Animals and reproductive management

Lactating Holstein dairy cows on a commercial dairy farm comprising approximately 1600 lactating cows located in south-central Wisconsin were enrolled into this study from 20 September 2005 to 23 May 2006. Cows were housed in free-stall barns with *ad libitum* access to water and were fed a total mixed ration consisting of corn silage and alfalfa silage with corn and soybean concentrate. Cows assigned to the study were coded by treatment, and lists for scheduled injections and pregnancy examinations for individual cows were generated weekly using a commercial on-farm computer software program (Dairy Comp 305, Valley Agricultural Software, Tulare, CA, USA). This program also was used to track and record treatment groups, reproductive outcomes, individual cow events, and monthly milk production records for each cow enrolled in the experiment. Data from ‘‘cowfile’’ archives were transferred into a computer spreadsheet program (Microsoft Excel 2002, Microsoft Corporation, Redmond, WA, USA) for organization and manipulation of data before statistical analysis using SAS (SAS Institute Inc., Cary, NC, USA).

2.2. Identification of anovular cows

Cows (n = 1047) were allocated weekly to breeding groups, each of which included cows that had calved within a given calendar week, but had not yet received a first postpartum TAI (range = 15–47 cows/group). All cows received a hormonal synchronization protocol (Presynch + Ovsynch) using im injections of 100 μg of GnRH (2 mL Cystorelin; Merial Ltd., Duluth, GA, USA) and 25 mg of $PGF_{2\alpha}$ (5 mL of Lutalyse; Pfizer Animal Health, New York, NY, USA) before first postpartum TAI, as follows: $PGF_{2\alpha}$ (Days 45 $\pm$ 3 and 59 $\pm$ 3), GnRH (Day 71 $\pm$ 3), $PGF_{2\alpha}$ (Day 78 $\pm$ 3), GnRH 54 h after $PGF_{2\alpha}$, followed by TAI 16 h later (Day 81 $\pm$ 3 postpartum).

Of the 1047 cows examined at the first GnRH injection of Presynch + Ovsynch using transrectal ultrasonography, 190 (18%) were classified as anovular based on the absence of a CL, using the method of Silva et al. [6]. Briefly, all cows were presynchronized with two injections of $PGF_{2\alpha}$ given 14 d apart, with the second injection 12 d before the first GnRH injection of Ovsynch. The rationale was that cycling cows receiving Presynch treatment would be expected to have a midcycle CL at the first GnRH injection of Ovsynch. When the ultrasound method of Silva et al. [6] was compared to a previously published method using two low ($\leq$1.0 ng/mL) serum P_4 samples collected at the second $PGF_{2\alpha}$ and first GnRH injections of Presynch + Ovsynch to classify cows as anovular, the ultrasound method was found to have 85.7% sensitivity and 87.7% specificity. Only cows classified as anovular were randomized to receive: (1) no further treatment after TAI (Control); or (2) 100 μg GnRH 4 d after AI (GnRH treated). A body condition score measured on a scale of one to five, with one being emaciated and five being obese [12], was assigned to anovular cows at the time of diagnosis.

2.3. Assessment of ovarian structures, blood sampling, and pregnancy diagnosis

Ultrasound examinations, blood sampling, hormone injections, and body condition scoring were conducted while cows were restrained in feed line headlocks after the morning milking. All ovarian structures $\geq$5 mm in diameter and the ovary on which they were located were recorded at the first GnRH and $PGF_{2\alpha}$ injections of Ovsynch, as well as 4 and 11 d after TAI, using an ultrasound scanner equipped with a transrectal 4.5–8.5 MHz linear-array transducer (Easi-Scan; BCF technology Ltd., Livingston, Scotland, UK). For each ovarian structure, diameter was estimated from a single frozen image of the apparent maximal diameter of the structure using on-screen background gridlines resulting in squares with 10 mm sides, as described previously [13].

Blood samples were collected via venipuncture of the median caudal vein or artery into evacuated tubes (Vacutainer; BD, Franklin Lakes, NJ, USA) at the first GnRH and the $PGF_{2\alpha}$ injections of Ovsynch, and again 4 and 11 d after TAI for later analysis of serum P_4. Blood samples were allowed to clot for 24 h at 4 °C, centrifuged (1935 × *g* for 15 min), and serum was harvested and stored at −20 °C until assayed for P_4 using a solid-phase, no-extraction RIA (Coat-a-Count Progesterone, Diagnostic Products Corporation, Los Angeles, CA, USA). Mean assay sensitivity was 0.09 ng/mL, and intra- and inter-assay coefficients of variation were 4.0 and 12.5%, respectively.

Pregnancy diagnosis was conducted by the herd veterinarian, with visualization of a CL, fluid-filled uterine horn, and presence of a conceptus used as positive indicators of pregnancy 31 d after TAI, using ultrasonography as described previously [14]. The number of cows diagnosed pregnant to TAI expressed as a percentage of cows within that treatment group receiving TAI was defined as pregnancies per AI.

2.4. Assessment of ovulatory response to Ovsynch

To determine if cows synchronized an ovulation after the second GnRH injection of Ovsynch, a combination of ultrasound scanning results from 4 and 11 d after TAI and serum P_4 4 d after TAI were used. Cows lacking a CL both 4 and 11 d after TAI ($n = 9$) were considered to have failed to ovulate to the second GnRH injection of Ovsynch. Logistical regression was used to evaluate the effect of serum P_4 4 d after TAI (Fig. 1). The greatest P_4 concentration 4 d after TAI for a cow diagnosed pregnant 31 d after TAI was 2.83 ng/mL, and the predicted probability of pregnancy for cows ($n = 20$) with P_4 above 2.83 ng/mL was <5%. Based on these results, the 20 cows with $P_4 > 2.83$ ng/mL 4 d after TAI were considered to have failed to regress their CL in response to the $PGF_{2\alpha}$ injection of Ovsynch, or ovulated a follicle before the second GnRH injection of Ovsynch. Overall, 29 cows failed to synchronize to Ovsynch and were not studied beyond the analysis of overall fertility.

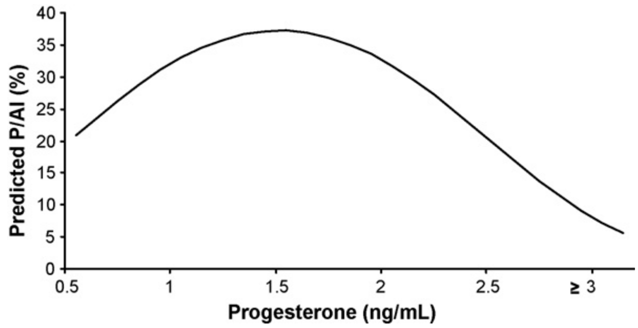

Fig. 1. Serum progesterone (P_4) concentration 4 d after timed AI (TAI) for all cows ($n = 146$) and the predicted probability of pregnancy. Because there were only 17 cows with P_4 >3.0 ng/mL, the final category for analysis was $P_4 \geq 3$ ng/mL. There was a quadratic effect ($P < 0.01$) of serum P_4 4 d after TAI on pregnancy status 31 d after TAI. The lowest serum P_4 concentration for a cow diagnosed pregnant was 0.19 ng/mL, whereas the greatest was 2.83 ng/mL. There were nine cows lacking a CL at 4 and 11 d after TAI that were considered to have failed to ovulate to the second GnRH injection of Ovsynch. There were also 20 cows with $P_4 > 2.83$ ng/mL 4 d after TAI, and none of these cows were diagnosed pregnant 31 d after TAI. Because the predicted P/AI for these cows was also <5%, they were classified as failing to synchronize to Ovsynch due to incomplete luteal regression or ovulation before the second GnRH injection of Ovsynch.

2.5. Experimental design and statistical analyses

All experiments were conducted using a randomized complete block design [15]. Each week at the first GnRH injection of Ovsynch, anovular cows within a weekly breeding group were blocked according to parity (primiparous vs. multiparous). Within each block, cows were assigned randomly to each of two treatments (Control vs. GnRH Treated) after first postpartum TAI.

To evaluate the effect of treatment and pregnancy status on serum P_4 concentration 4 and 11 d after TAI, continuous data were analyzed using PROC MIXED for repeated measures (SAS Institute Inc., Cary, NC, USA). Only cows in which ovulation was synchronized and that had a luteal phase >11 d were used for this analysis. Variables included in the model were treatment, day, pregnancy status, and the interactions of treatment × day and pregnancy status × day. Differences between classes for follicle size (<10 vs. ≥10 mm) and P_4 (<1 vs. ≥1 ng/mL) were analyzed using a Chi-square test for specified proportions using the FREQ procedure of SAS.

Dichotomous data (P/AI, short luteal phase, etc.) were analyzed using PROC LOGISTIC of SAS (SAS Institute Inc.). A multivariate logistical regression model was developed to analyze the effects of the categorical variables treatment, parity (primiparous vs. multiparous), AI technician, and the continuous variable BCS on P/AI.

In addition, all two-way interactions of the explanatory variables with treatment on P/AI and pregnancy loss also were included. The effect of AI sire was not included in the model, but the 21 sires used for AI were distributed randomly among treatments. Follicle size and P_4 concentration versus probability of pregnancy was analyzed, with a binomial distribution assumed for the response variable pregnancy. One- and two-way interactions with expected effects were included in the model. Using estimates from the fixed effects obtained in the logistic regression analysis, the probability of pregnancy was calculated using the formula:

$$P = 1/1 + e^{-(b0 + b1X1 + b2X2 + \cdots + biXi)}$$

All multivariate logistical regression models were constructed using a backward selection procedure with treatment retained as a fixed factor in each of the models. A Wald statistic criterion of $P < 0.15$ was set for including variables in the model. Data are presented as percentages and proportions with P values for main effects and interactions derived from the multivariate logistical regression analysis.

3. Results

3.1. Synchronization of ovuation

Initially, 1047 cows were examined ultrasonographically at the time of the first GnRH injection of Ovsynch to determine the presence or absence of a CL, and 190 cows (18.1%) lacked a CL at this time and were classified as anovular. Retrospective analysis indicated that 20 cows without a detectable CL had $P_4 \geq 1$ ng/mL at the first GnRH of Ovsynch, and these cows were removed from the data set. Interestingly, 17 of these cows had ovarian structures $\geq$25 mm in diameter that may have been misclassified as follicular rather than luteinized cysts. Moreover, after the first GnRH injection of Ovsynch, four cows were coded by herd personnel as "do not breed," five cows did not receive TAI after the Presynch + Ovsynch protocol, three cows were sold or died, one cow received an incorrect hormonal synchronization injection sequence, and one cow did not receive a pregnancy diagnosis after enrollment into study. After elimination of these cows, the final data set included a total of 156 anovular cows. Not all of the 156 remaining anovular cows were located at the time of data collection, and all experimental units may not match across days due to missing cows or blood samples.

Because ultrasound examinations and blood sampling occurred at weekly intervals, a combination of data from 4 and 11 d after TAI was used to determine ovulation after the second GnRH injection of Ovsynch. A total of nine cows were determined to have failed to ovulate as indicated by no detectable luteal tissue and low P_4 at 4 and 11 d after TAI. At 4 d after TAI, the lowest P_4 concentration for a cow identified pregnant at 31 d after TAI was 0.19 ng/mL, whereas the highest P_4 concentration 4 d after TAI for a cow identified pregnant at 31 d after TAI was 2.83 ng/mL and regression analysis predicted a probability of pregnancy of <5% for cows with $P_4 > 2.83$ ng/mL (Fig. 1). Based on these observations, we concluded that the 20 cows with $P_4 > 2.83$ ng/mL (range = 2.86–6.38) 4 d after TAI either failed to regress their CL in response to the $PGF_{2\alpha}$ injection of Ovsynch or ovulated a follicle before the second GnRH injection of Ovsynch. Overall, 81.4% (127/156) of cows synchronized an ovulation after the second GnRH injection of Ovsynch, and synchronization rates for control and G4 cows were 85.9% (73/85) and 76.1% (54/71), respectively.

3.2. Response to GnRH 4 d after TAI

Overall, the ovulatory response to GnRH treatment 4 d after TAI was 51.0% (25/49; Table 1). The size of the largest ovarian follicle was recorded 4 d after TAI, and follicle size was categorized as <10 mm or ≥10 mm for G4 cows. There was no difference ($P = 0.43$) in the proportion of cows ovulating in response to GnRH based on follicle size class (40%, 4/10 vs. 53.4% 21/39). In addition, P/AI did not differ ($P = 0.24$) between cows that ovulated in response to GnRH treatment (36.0%; 9/25) and those that failed to ovulate to GnRH treatment (20.8%; 5/24).

Follicle size at the time of G4 treatment was monitored to determine the proportion of cows with an ovulatory follicle present. For cows receiving the GnRH treatment, 20.4% (10/49) had follicles <10 mm in diameter at the time of treatment, with four of those cows ovulating follicles 8–9 mm in diameter. For cows

Table 1
Number of Holstein cows ovulating to GnRH treatment 4 d after timed AI (TAI) and the effect of ovulation on pregnancies per AI (P/AI) at 31 d after TAI

Response to GnRH treatment	No.	P/AI, % (No./No.)	*P*-value
Ovulation	25	36.0 (9/25)	0.24
No ovulation	24	20.8 (5/24)	

Ovulation to GnRH treatment 4 d after TAI is unknown for five cows due to missing cows or missing samples at the time of evaluations ($n = 2$) and because three cows had a luteal phase <11 d.

Table 2
Effect of treatment with GnRH 4 d after timed AI on the proportion of anovular lactating Holstein cows with a short luteal phase and pregnancies per AI (P/AI)[a]

Variable	Treatment		*P*-value
	Control[b]	GnRH treated[c]	
No.	85	71	–
Proportion of cows synchronized	85.9 (73/85)	76.1 (54/71)	–
Proportion of synchronized cows with a short luteal phase (<11 d)	11.3 (8/71)	5.6 (3/54)	0.25
P/AI, % (No./No.)			
All cows	25.9 (22/85)	22.5 (16/71)	0.63
Synchronized cows	30.1 (22/73)	29.6 (16/54)	0.89

Experimental units may not match within columns due to missing cows or missing samples at the time of evaluations.
[a] Cows were synchronized before first postpartum TAI as follows: $PGF_{2\alpha}$ (Days 45 ± 3 and 59 ± 3), GnRH (Day 71 ± 3), $PGF_{2\alpha}$ (Day 78 ± 3), GnRH 54 h after $PGF_{2\alpha}$ followed by TAI 16 h later (Day 81 ± 3 postpartum).
[b] Cows received no further treatment after timed AI.
[c] Cows received 100 μg GnRH 4 d after timed AI.

with follicles ≥10 mm, 53.8% (21/39) ovulated in response to the GnRH treatment.

Contrary to our hypothesis, P/AI of cows ovulating to the GnRH treatment (36%; 9/25) did not differ ($P = 0.24$) from that of cows failing to ovulate to the GnRH treatment (21%; 5/24).

3.3. Effect of treatment on fertility

Percentages and proportions for P/AI for cows receiving Control versus GnRH treatments are shown (Table 2). Treatment did not affect P/AI for control (25.9%; 22/85) or GnRH treated (22.5%; 16/71) cows, or for control (30.1%; 22/73) and G4 (29.6%; 16/54) cows that were synchronized.

The effect of P_4 class (<1 vs. ≥1 ng/mL) at the $PGF_{2\alpha}$ injection of Ovsynch is shown (Table 3). Cows with $P_4 < 1$ ng/mL had fewer ($P < 0.01$) P/AI (11.6%, 5/43) than cows with $P_4 \geq 1$ ng/mL (40.8%, 31/76), regardless of treatment ($P = 0.76$). Cows with $P_4 \geq 1$ ng/mL treated with G4 had similar ($P = 0.57$) P/AI (44.8%, 13/29) compared to control cows (38.3%, 18/47). There was a quadratic effect ($P = 0.02$) of P_4 at the $PGF_{2\alpha}$ injection of Ovsynch on predicted P/AI (Fig. 2).

It is noteworthy that cows with $P_4 \geq 1$ ng/mL at the $PGF_{2\alpha}$ injection of Ovsynch had greater fertility than cows with $P_4 < 1$ ng/mL, indicating the importance of P_4 at the $PGF_{2\alpha}$ injection of Ovsynch for anovluar cows. Linear regression was also used to determine the effect of P_4 at the $PGF_{2\alpha}$ injection of Ovsynch. Because there were too few cows with $P_4 > 3$ ng/mL ($n = 12$) for this analysis, these cows were categorized as having $P_4 \geq 3$ ng/mL. Based on the analysis, 2.4 ng/mL P_4 at the $PGF_{2\alpha}$ injection of Ovsynch resulted in the greatest predicted P/AI (42.2%; Fig. 2). A 50% reduction in predicted P/AI occurred when P_4 at the $PGF_{2\alpha}$ injection of Ovsynch was <1 ng/mL (20.9% P/AI), which supports our use of P_4 classes of <1 and ≥1 ng/mL.

3.4. Effect of treatment on the proportion of cows ovulating multiple follicles

There was no difference ($P = 0.78$) between P_4 classes in the proportion of cows ovulating more than

Table 3
Effect serum progesterone (P_4) at the $PGF_{2\alpha}$ injection of Ovsynch on multiple ovulations to the second GnRH injection of Ovsynch, the proportion of cows with a short luteal phase (<11 d), and pregnancies per AI (P/AI) for anovular lactating Holstein cows receiving synchronization of ovulation and timed AI

	Multiple ovulations, % (No./No.)	Proportion of cows with a short luteal phase (<11 d)	P/AI, % (No./No.)		*P*-value
			Control	GnRH treated	
P_4 class					
$P_4 < 1$ ng/mL	30.2 (13/43)	16.3[a] (7/43)	13.0[a] (3/23)	10.0[a] (2/20)	0.76
$P_4 \geq 1$ ng/mL	27.7 (20/72)	5.4[b] (4/74)	38.3[b] (18/47)	44.8[b] (13/29)	0.57

Experimental units may not match among columns due to missing cows or missing samples at the time of evaluations. [a,b]Within a column, proportions differ ($P \leq 0.05$).

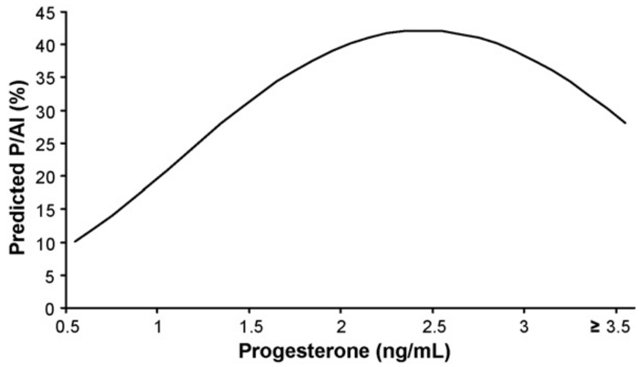

Fig. 2. Predicted pregnancies per AI (P/AI) for synchronized cows ($n = 119$) based on serum progesterone (P_4) concentration at the time of the $PGF_{2\alpha}$ injection of Ovsynch. Only 12 cows had $P_4 > 3.5$ ng/mL, and these cows were included in the $P_4 \geq 3.5$ ng/mL category. There was a quadratic relationship ($P = 0.02$) between serum P_4 at the time of the $PGF_{2\alpha}$ injection of Ovsynch and P/AI 31 d after timed AI.

one follicle to the second GnRH injection of Ovsynch (30.2%; 13/43 vs. 27.7%; 20/72).

3.5. Effect of treatment on the proportion of cows with a short luteal phase

The proportion of cows with a luteal phase <11 d is shown in Table 2, and was 8.8% (11/125). There was no difference ($P = 0.25$) in the proportion of cows with a short luteal phase between control (11.3%; 8/71) and GnRH treatment (5.6%; 3/54). The proportion of cows with a short luteal phase (<11 d) was greater ($P = 0.05$) for cows with $P_4 < 1$ ng/mL (16.3%, 7/43) at the $PGF_{2\alpha}$ injection of Ovsynch than cows with $P_4 \geq 1$ ng/mL (5.4%, 4/74), re-emphasizing the importance of P_4 exposure before TAI (Table 3).

3.6. Effect of treatment and pregnancy status on P_4

Serum P_4 at 4 and 11 d after TAI based on treatment and pregnancy status 31 d after TAI are depicted in Figs. 3 and 4, respectively. Serum P_4 4 d after TAI did not differ ($P = 0.69$) between control (1.11 ± 0.08 ng/mL) and GnRH treated cows that ovulated (1.17 ± 0.13 ng/mL), between control and GnRH treated cows that did not ovulate ($P = 0.16$; 0.89 ± 0.14 ng/mL), or between GnRH treated cows that ovulated and GnRH treated cows that did not ovulate ($P = 0.13$). For serum P_4 at 4 d after TAI, there was no difference ($P = 0.75$) between pregnant and non-pregnant cows (1.14 ± 0.10 vs. 1.05 ± 0.08 ng/mL).

At 11 d after TAI, serum P_4 did not differ ($P = 0.39$) between control cows (4.9 ± 0.27 ng/mL), and GnRH treated cows that ovulated in response to the GnRH treatment (5.29 ± 0.38 ng/mL). Cows failing to ovulate to G4 treatment tended ($P = 0.06$) to have lower P_4 (3.90 ± 0.33 ng/mL) than control cows, and had significantly lower ($P = 0.02$) P_4 than GnRH treated cows that ovulated to GnRH treatment. Cows diagnosed pregnant 31 d after TAI had greater ($P < 0.01$) serum P_4 11 d after TAI (5.42 ± 0.28 ng/mL) compared to cows diagnosed non-pregnant (4.43 ± 0.25 ng/mL; Fig. 4).

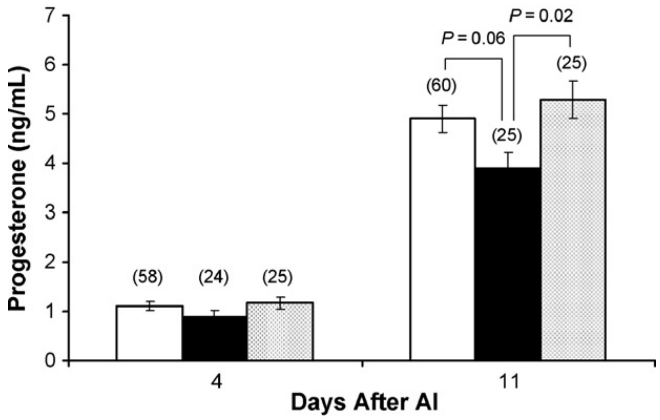

Fig. 3. Mean (±S.E.M.) serum progesterone concentration (ng/mL) 4 and 11 d after timed AI (TAI) for anovular lactating Holstein cows. Numbers in parentheses represent the number of cows per treatment. Cows received no treatment (open bars) or 100 μg of GnRH 4 d after TAI. Dappled bars represent cows ovulating to the GnRH injection 4 d after TAI, whereas black bars represent cows that failed to ovulate to the GnRH injection 4 d after TAI. Experimental units may not match between days after AI due to missing cows or missing samples at the time of evaluations.

4. Discussion

This study tested the hypothesis that treatment of anovular cows with GnRH 4 d after first postpartum TAI

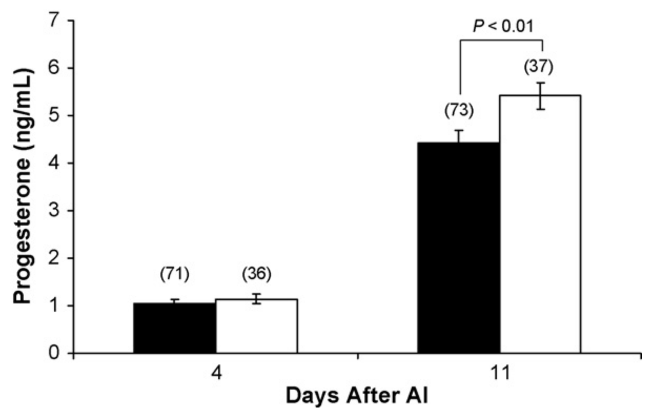

Fig. 4. Mean (±S.E.M.) serum progesterone concentration (ng/mL) 4 and 11 d after timed AI (TAI) for anovular lactating Holstein cows diagnosed pregnant (open bars) or not-pregnant (black bars) 31 d after TAI. Numbers in parentheses represent the number of cows per treatment. Experimental units may not match between days after AI due to missing cows or missing samples at the time of evaluations.

would improve P/AI. This hypothesis was based on the results of an earlier study in which cows treated with GnRH 5 d after TAI tended to improve fertility due to a treatment by cyclicity interaction [11]. However, our hypothesis was not supported. There were two important differences between the current study and that of Sterry et al. [11]: (1) all anovular cows in the previous study were exposed to P_4 via a CIDR between the first GnRH injection and the $PGF_{2\alpha}$ injection of Ovsynch; (2) the interval from the second GnRH injection and GnRH treatment after TAI was reduced by 0.5 d.

One of the concerns regarding GnRH treatment as early as 4 d after TAI (4.5 d after the second GnRH injection of Ovsynch) is whether a follicle of ovulatory size (≥10 mm in diameter [16]) would be present. When a limited number of cows were synchronized with Ovsynch and treated with GnRH 5 d after TAI, 70% (7/10, [11]) and 100% (12/12, [17]) of the cows ovulated. Likewise, 86.2% (175/203) of cows treated with hCG 5 d after AI to detected estrus had multiple CL, compared to 23.2% (47/203) for control cows [18].

While follicle diameter <10 mm is a known to result in a poor ovulation risk to GnRH treatment, the majority of cows in the present study had follicles ≥10 mm. Thus, small follicle size 4 d after TAI alone cannot explain the poor ovulation risk to GnRH treatment. Although follicular size and number could be determined, one of the limitations with once-weekly ultrasound examinations was that we were unable to distinguish whether follicles were developing or atretic at the time of GnRH treatment. Thus, it is possible that some cows with a follicle >10 mm in diameter in the present study lacked a follicle with ovulatory capacity.

Although P/AI was 15 percentage points greater for GnRH treated cows that ovulated to GnRH treatment compared to those cows that failed to ovulate (36 vs. 21%), a total of 140 experimental units per treatment would have been required to detect this difference using a two-tailed test (95% confidence, 80% power; Win Episcope 2.0). Due to the low numbers of experimental units in the present study (≤25 when G4 cows were subdivided into classes that ovulated and failed to ovulate to treatment), it is also important to consider the possibility of Type II errors when interpreting the results of the present study. Cows treated with hCG that had multiple CL exhibited greater P/AI (44%, 77/175), compared to cows with a single CL after treatment (4%, 1/25; [18]). In addition, cows treated with GnRH 7 d after AI that ovulated had greater P/AI compared to cows failing to respond to GnRH on Day 7 [19]. It is important to note that previous results showing greater fertility to treatments 5 or 7 d after AI were for cycling cows or cows in which cyclicity status was unknown, whereas the present study included only cows classified as anovular.

The findings of this study disagreed with previous results in which 100 μg of GnRH administered 5 d after first postpartum TAI following Presynch + Ovsynch tended to improve P/AI for anovular, but not cycling cows [11]. Conversely, hCG 5 d after AI had no effect on cows synchronized with a CIDR and estradiol benzoate that were not observed in estrus and had no palpable CL; however, only cows observed in estrus were submitted for AI [20]. Thus, the synchronization treatment used to submit cows for AI may have resolved the anovular condition, which may explain the lack of a treatment effect.

Incorporation of a CIDR between the first GnRH injection and the $PGF_{2\alpha}$ injection of Ovsynch improved P/AI for both anovular dairy [7] and beef cows [21] failing to ovulate in response to the first GnRH injection of Ovsynch. Thus, cows with $P_4 < 1$ ng/mL at the $PGF_{2\alpha}$ injection of Ovsynch have poorer fertility to TAI, and may not benefit from the GnRH treatment. It is important to note that a key difference between the current study and that of Sterry et al. [11] is that cows lacking a CL in the study by Sterry et al. [11] were given an intravaginal device containing P_4 (CIDR) between the first GnRH and $PGF_{2\alpha}$ injections of Ovsynch. Inclusion of cows with $P_4 < 1$ ng/mL at the $PGF_{2\alpha}$ injection of Ovsynch may explain why anovular cows not receiving a CIDR did not have greater P/AI after G4 treatment, whereas cows exposed to P_4 via a CIDR device during Presynch + Ovsynch and treated with GnRH 5 d after TAI tended to have improved fertility. Unfortunately, there were too few cows with $P_4 \geq 1$ ng/mL at the $PGF_{2\alpha}$ injection of Ovsynch (47 control and 29 GnRH cows) to have adequate statistical power to detect treatment differences in this subgroup of cows.

No difference between P_4 classes in the proportion of cows ovulating more than one follicle was surprising, because other reports indicate low P_4 before spontaneous or induced ovulations results in a greater multiple ovulation rate. Cows with a spontaneous first postpartum ovulation between 70 and 100 d postpartum had a multiple ovulation rate of 46.3% [8]. When anovular cows were synchronized with Ovsynch the multiple ovulation rate following the first GnRH injection was 41%, but only 13% following the second GnRH injection [5]. Stevenson et al. [7], though, also reported no difference between high and low P_4 classes in the proportion of cows ovulating more than one follicle following the second GnRH injection of Ovsynch (15.4 vs. 16.5%).

Although treatment reduced the proportion of cows with a short luteal phase by five percentage points, a total of 486 experimental units per treatment would have been required to detect this difference using a two-tailed test (95% confidence, 80% power; Win Episcope 2.0). The proportion of anovular cows synchronized with Ovsynch with a luteal phase <11 d was less than expected based on the 23% previously reported [5]. Although GnRH treated cows had half the incidence of luteal phases <11 d (5.6 vs. 11%), this difference was not significant ($P = 0.25$).

It is intriguing that ovulatory response to GnRH treatment did not significantly improve fertility in anovular cows, since they may benefit from both greater P_4 produced by an accessory CL, and reduced estradiol-17β (E_2) caused by ovulation of an ovarian follicle(s). Removal of ovarian follicles by electrocautery prolongs luteal function [22,23]. In ewes, E_2 up regulates uterine oxytocin receptors and stimulates pulsatile release of $PGF_{2\alpha}$ [24]. This may be particularly important in anovular cows which have not been exposed to P_4 and may lack sufficient uterine P_4 receptors to prevent early luteolysis [25].

Cows diagnosed pregnant 31 d after TAI having greater serum P_4 11 d after TAI agrees with the findings of Gümen et al. [5] for cycling cows submitted for TAI after Ovsynch, in which P_4 at 11 d but not 4 d after TAI, was greater for pregnant than not-pregnant cows. Mann and Lamming [26] observed that cows lacking an embryo 16 d after AI had a delayed increase and lower peak concentration of P_4 after AI than cows with embryos, further emphasizing the importance of post AI P_4. However, P_4 before 11 d after TAI has been shown to be important and is not reflected in these data, because at 5 d after AI embryo development past the eight-cell stage was associated with greater P_4 and lower insulin, indicating that P_4 may have a direct effect on the uterine environment [27].

The ineffectiveness of GnRH treatment to increase serum P_4 was not unexpected because only about half of the GnRH treated cows responded to GnRH treatment by ovulating a follicle. Despite this observation, previous studies have reported increased P_4 by 11 d after AI for cows treated with GnRH 5 d after AI [17,28]. Explanations for the lack of a treatment effect, particularly in cows ovulating in response to the GnRH treatment, are not clear at this time.

In conclusion, of the 1047 lactating dairy cows being submitted for first postpartum TAI, 190 cows lacked a CL at the first GnRH injection of Presynch + Ovsynch and were diagnosed as anovular; 156 of these anovular cows were further analyzed. Contrary to our hypothesis, treatment of anovular lactating dairy cows with GnRH 4 d after TAI failed to improve P/AI or reduce the proportion of cows with a short luteal phase. Only half (51%) of the cows receiving GnRH after TAI ovulated in response to GnRH treatment, and there was no significant effect on P/AI based on the ovulatory response to GnRH treatment. Although cows diagnosed pregnant 31 d after TAI had greater serum P_4 11 d after TAI, treatment with GnRH failed to increase P_4 by 11 d after TAI. Although treatment of anovular cows with GnRH 4 d after TAI did not improve fertility, variation among cows in serum P_4 at the $PGF_{2\alpha}$ injection of Ovsynch dramatically affected P/AI and the proportion of anovular cows with a short luteal phase. Considering the labor requirement to identify anovular cows, variability of post TAI treatment response, and the importance of P_4 at the $PGF_{2\alpha}$ injection of Ovsynch, we suggest future studies should take the approach of improving fertility of anovular cows through improved synchronization protocols rather than post-TAI treatments.

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