Strategic use of gonadotrophin-releasing hormone (GnRH) to increase pregnancy rate and reduce pregnancy loss in lactating dairy cows subjected to synchronization of ovulation and timed insemination


Department of Large Animal Clinical Sciences, College of Veterinary Medicine, University of Florida, Gainesville, FL 32610, USA
Department of Animal Sciences, University of Florida, Gainesville, FL 32610, USA
North Florida Holsteins, Inc., Bell, FL 32619, USA

Received 2 April 2004; accepted 20 May 2004

Abstract

The objective of this study was to determine the effect of GnRH (100 μg i.m.) treatment 5 and 15 days after timed insemination (TAI) on pregnancy rate and pregnancy loss in lactating dairy cows subjected to synchronization of ovulation. The study included 831 lactating dairy cows subjected to a Presynch–Ovsynch protocol for first service. On the day of TAI (Day 0), cows were randomly assigned to one of four experimental groups. Cows in Group 1 (n = 214) were treated with GnRH on Day 5; cows in Group 2 (n = 209) were treated with GnRH on Day 15; cows in Group 3 (n = 212) were treated with GnRH on both Day 5 and Day 15; cows in Group 4 (n = 196) were not treated. Pregnancy rate was evaluated at Day 27 and Day 45 after TAI. The interestrus interval and the proportion of cows

Portions of the results presented in this manuscript were reported in the Proceedings of the 83rd Annual Meeting, Conference of Research Workers in Animal Diseases, November 10–12, 2002, St. Louis, MO, USA, Abstract 216.

* Corresponding author. Tel.: + 1 352 392 4700x5641
E-mail address: archbaldl@mail.vetmed.ufl.edu (L.F. Archbald).

0093-691X/$ – see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.theriogenology.2004.05.020
diagnosed not pregnant based on expression of estrus and insemination before pregnancy diagnosis on Day 27 were determined. The results of this study are: (1) GnRH treatment on Day 5 or Day 15 did not increase pregnancy rate, or reduce pregnancy loss between Day 27 and Day 55 after TAI; (2) cows treated with GnRH on both Day 5 and Day 15 had a lower ($P < 0.01$) proportion of cows diagnosed not pregnant based on expression of estrus before ultrasonography on Day 27 (26.5%) compared to control cows (52.9%), and these cows had an extended ($P = 0.05$) interestrus interval (23.4 days vs. 21.5 days); and (3) GnRH treatment on both Day 5 and Day 15 after TAI reduced pregnancy rate on Day 27 (36.8% vs. 44.4% for control cows; $P < 0.03$) and Day 55 (28.3% vs. 36.2% for control cows; $P < 0.01$). Therefore, strategies to stimulate CL function using multiple doses of GnRH during the luteal phase need to consider potential negative effects.

# 2004 Elsevier Inc. All rights reserved.

Keywords: Dairy cows; Timed insemination; Pregnancy loss; GnRH

1. Introduction

Pregnancy rate for 21 days periods, which is the product of conception rate and estrus detection rate, is the most commonly used parameter to evaluate reproductive performance in dairy herds [1]. Synchronization of ovulation with TAI (without detection of estrus) is commonly used in lactating dairy cows to increase pregnancy rate [2–4]. However, pregnancy loss in cows subjected to TAI appears to be as high as that observed in cows inseminated at detected estrus [5,6].

After fertilization, corpus luteum (CL) development, follicular growth, and progesterone ($P_4$) and estrogen concentrations influence embryo survival and CL maintenance [7]. Corpus luteum development results in secretion of $P_4$, which influences embryo development, interferon-τ production, and inhibition of the luteolytic cascade [8]. Destruction of ovarian follicles by electrocauterization during mid-cycle reduced estrogen concentrations, extended luteal function [9], increased CL weight [10] and reduced the efficacy of PGF$_{2\alpha}$-induced luteolysis [11].

The detrimental effect of low $P_4$ and high estrogen concentrations on early embryo development may be exacerbated in high-producing dairy cows subjected to synchronization of ovulation. Lactating dairy cows have an increased feed intake that results in increased liver blood flow and steroid metabolism [12] that results in low $P_4$ concentrations. Low concentrations of $P_4$ during diestrus increase luteinizing hormone (LH) pulses [13], which stimulate follicular growth [14,15] resulting in elevated concentration of estradiol. In fact, the lifespan of the dominant follicle is extended and its size increased in lactating compared to nonlactating dairy cows [16]. In an experiment using timed insemination, when timing between GnRH administration for induction of ovulation and follicular development was altered, ovulation resulted in formation of a smaller corpus luteum with reduced production of progesterone [17–19].

Early embryonic mortality, indicated by cows returning to estrus before 24 days after insemination, is approximately 20.5–43.6%, and late embryonic mortality with cows returning to estrus after 24 days is approximately 8.0–17.5% [20]. Late embryonic mortality in cows subjected to synchronization of ovulation protocols was 14.6% measured between pregnancy diagnosis on Days 32 and 74 after TAI [21].
Since LH is luteotrophic in the bovine [22], strategies involving administration of bovine LH, LH-containing preparations, human chorionic gonadotropin (hCG), or GnRH have been used to stimulate CL function [23–27]. The ovulation of dominant follicles and formation of accessory CL increase $P_4$ and reduce estrogen concentrations [28–30]. There is a paucity of information available on the effect of GnRH administered coincident with the presence of the dominant follicle of the first (Day 5) and second (Day 15) follicular wave in cows subjected to synchronization of ovulation and TAI.

The hypothesis of this study was that treatment of cows with GnRH at specific times after insemination will increase pregnancy rate and reduce pregnancy loss in lactating dairy cows subjected to synchronization of ovulation and TAI. The objectives of this study were to compare pregnancy rates (PR) at Days 27 and 55, and pregnancy loss between Day 27 and Day 55 in lactating dairy cows treated with GnRH 5 and (or) 15 days after TAI.

2. Materials and methods

2.1. Study population

This study was conducted during the period of November 2001 to April 2002 in a large commercial dairy herd (approximately 3000 milking cows) in north-central Florida. All reproductive, health and management records were computerized using a cow-side computer system. Cows were routinely vaccinated against bovine virus diarrhea, infectious bovine rhinotracheitis, bovine respiratory syncytial virus, parainfluenza virus, leptospirosis, campylobacteriosis, clostridiosis, and Gram-negative organisms according to recommendations by attending veterinarians. Cows were housed in free stall barns and dry lots and fed a total mixed ration (TMR) three times daily. The TMR was formulated to meet or exceed requirements for lactating cows [31]. Cows were milked three times a day, and had a rolling herd average for milk production of 10,700 kg. Beginning at 60 days postpartum, cows received bST (Posilac®, 500 mg sometribove zinc, subcutaneously; Monsanto, St Louis, MO, USA) every 14 days during the entire period of the study. The reproductive management employed in this herd consisted of a voluntary waiting period of 100 days for multiparous cows, and 140 days for primiparous cows. All cows were subjected to Presynch–Ovsynch program [21] for first service.

The methods of estrus detection used in this study included a computerized system which used increased walking activity and an accompanying decrease in milk production (Afi milk®, S.A.E. Afikim, Kibbutz Afikim, Israel), as well as daily visual observations of the cows. A specific individual was assigned the duty of observing these cows for estrus during each 8-h shift. Thus, the cows were continuously observed for estrus.

2.2. Study design

A total of 831 reproductively normal (cows with reproductive abnormalities such as metritis, pyometra, uterine–ovarian adhesions were excluded) cows was used in this study. Multiparous cows were treated with one luteolytic dosage of PGF$_{2a}$ (25 mg, i.m.; 5 mL of Lutalyse®, Sterile Solution, Pfizer Animal Health, New York, NY, USA) on Day 64 and
Day 78 PP, GnRH (100 µg, i.m.; 2 mL of Cystorelin®, Merial Limited, Iselin, NJ, USA) on Day 90 PP, PGF$_{2α}$ on Day 97 PP, GnRH on Day 99 PP, and time inseminated 16 h later (Day 100 PP; Experimental Day 0). Primiparous cows were treated with PGF$_{2α}$ (25 mg, i.m.) on Day 104 PP and Day 118 PP, GnRH (100 µg, i.m.) on Day 130 PP, PGF$_{2α}$ on Day 137 PP, GnRH on Day 139 PP, and time inseminated 16 h later (Day 140 PP; Experimental Day 0).

On Day 0, 831 primiparous and multiparous cows were assigned randomly to receive either GnRH on Day 5 or Day 15 after TAI. Sample size was calculated based on an expected difference in pregnancy rate of 5% for the main effects of GnRH on Day 5 or Day 15. Cows in Group 1 (n = 214) were treated with GnRH (100 µg, i.m.) on Day 5 post-TAI; cows in Group 2 (n = 209) were treated with GnRH (100 µg, i.m.) on Day 15 post-TAI; cows in Group 3 (n = 212) were treated with GnRH (100 µg, i.m.) on both Day 5 and Day 15 post-TAI; cows in Group 4 (n = 196) were not treated and served as controls.

Since there was an interest in determining any “seasonal effects” of treatment on reproduction in these cows, it was decided to divide the year into “favorable” and “nonfavorable” times of fertility. Previous research[32] has shown that pregnancy rates in Florida are higher during November, December, January, and February compared with other times of the year. Thus, the period of November to February was considered favorable, and March and April were considered unfavorable for optimum pregnancy rates. In addition, there was also an interest in determining the effect of parity and inseminator on pregnancy rates. Therefore, on Day 0, information concerning parity, time of year (season), and inseminator were obtained.

2.3. Pregnancy diagnosis

Pregnancy was determined 27 days after insemination using transrectal ultrasonography (5 MHz linear probe, Aloka 500V, Wallingford, CT, USA). Cows were classified as: (1) pregnant: presence of an embryo exhibiting heartbeats and surrounded by a fluid-filled cavity (allantoic) in the uterine lumen [33,34]; (2) questionable pregnant: absence of an embryo, but presence of intrauterine fluid and a corpus luteum on the ovaries; (3) not pregnant: cows that were inseminated at detected estrus before ultrasonography on Day 27 or diagnosed not pregnant by ultrasonography. On Day 55, pregnancy was reconfirmed by per rectum palpation of the uterus using previously described techniques and criteria [35].

2.4. Statistical analysis

Baseline comparisons for parity (1–3+), season (November to February and March/April), and inseminators (A–C) were carried out to establish comparability of groups using a $\chi^2$-test. A univariate analysis for the effect of group on pregnancy rates at Day 27 and Day 55 and pregnancy loss between Day 27 and Day 55 was conducted using $\chi^2$-test. A multivariate analysis was conducted to evaluate the main effect of GnRH on Day 5 and Day 15 and the interaction between GnRH on Day 5 and Day 15 on pregnancy rate and pregnancy loss. The model included the main effect of GnRH on Day 5, the main effect of GnRH on Day 15, the interaction between both GnRH on Day 5 and Day 15, as well as parity, season and inseminator to adjust for residual confounding using the backward
elimination procedure [36] in a logistic regression model (PROC GENMOD [37]). Variables remained in the model at \( P < 0.15 \). The proportion of cows diagnosed not pregnant based on insemination before Day 27 was analyzed by \( \chi^2 \). The interval (days) from first service to next insemination was analyzed by analysis of variance [38] using Proc GLM of SAS\textsuperscript{16}. Differences between groups with a \( P \)-value \( \leq 0.05 \) were considered significant and those with a \( P \)-value \( \leq 0.10 \) were considered to represent a tendency.

3. Results

There was no difference in the distributions of cows by parity, season and inseminator among the four groups (Table 1). In the univariate analysis, pregnancy rates on Day 55 tended \( (P < 0.06) \) to be lower in cows in Group 3 compared to Group 4, but there was no difference in pregnancy rate on Day 27 and pregnancy loss among groups (Table 2).

In the multivariate analysis, the interaction between treatment of cows with GnRH on Day 5 and Day 15 on pregnancy rate tended to be significant \( (P < 0.10) \). Administration of GnRH on Day 15 tended to decrease pregnancy rate on Day 27 only in cows previously treated with GnRH on Day 5. In addition, there was an effect \( (P < 0.01) \) of season on pregnancy rate. Therefore, the final model to evaluate pregnancy rates on Days 27 and 55 and pregnancy loss between Day 27 and Day 55 included group and season.

The number of cows pregnant on Day 27 and Day 55, the risk of nonpregnancy, adjusted odd ratios (AOR), 95% confidence interval (CI) and level of significance for all groups adjusting for season are shown in Tables 3 and 4, respectively. There was an increase in the risk of nonpregnancy on Day 27 (AOR = 1.4, 95% CI 0.9–2.1, \( P < 0.03 \)) and on Day 55 (AOR = 1.4, 95% CI 1.0–2.2, \( P < 0.01 \)) for cows in Group 3.

The number of cows which lost a pregnancy between Day 27 and Day 55, the risk of pregnancy loss, adjusted odd ratios, 95% confidence interval and level of significance for

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group 1 (GnRH Day 5)</th>
<th>Group 2 (GnRH Day 15)</th>
<th>Group 3 (GnRH Days 5 and 15)</th>
<th>Group 4 (Control)</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.67</td>
</tr>
<tr>
<td>1</td>
<td>31.8</td>
<td>31.6</td>
<td>25.5</td>
<td>32.1</td>
<td>63/196</td>
</tr>
<tr>
<td>2</td>
<td>34.1</td>
<td>34.4</td>
<td>35.4</td>
<td>30.6</td>
<td>60/196</td>
</tr>
<tr>
<td>3+</td>
<td>34.1</td>
<td>34.0</td>
<td>39.1</td>
<td>37.3</td>
<td>73/196</td>
</tr>
<tr>
<td>Season</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.96</td>
</tr>
<tr>
<td>November–February</td>
<td>53.3</td>
<td>53.6</td>
<td>51.9</td>
<td>51.5</td>
<td>101/196</td>
</tr>
<tr>
<td>March/April</td>
<td>46.7</td>
<td>46.4</td>
<td>48.1</td>
<td>48.5</td>
<td>95/196</td>
</tr>
<tr>
<td>Inseminator</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.80</td>
</tr>
<tr>
<td>A</td>
<td>41.6</td>
<td>42.1</td>
<td>44.3</td>
<td>47.9</td>
<td>94/196</td>
</tr>
<tr>
<td>B</td>
<td>28.5</td>
<td>28.7</td>
<td>29.7</td>
<td>28.1</td>
<td>55/196</td>
</tr>
<tr>
<td>C</td>
<td>29.9</td>
<td>29.2</td>
<td>25.9</td>
<td>20.1</td>
<td>47/196</td>
</tr>
</tbody>
</table>
Cows in Group 3 (GnRH treatment on both Day 5 and Day 15) had a lower proportion of cows diagnosed not pregnant based on expression of estrus and insemination before ultrasonography on Day 27, and these cows had an extended interestrus interval compared with cows in Group 4 (untreated controls). The proportion of cows diagnosed not pregnant based on expression of estrus and insemination before ultrasonography on Day 27 was 46.2% (49/106) for Group 1, 42.3% (48/113) for Group 2, 36.8% (78/212) for Group 3, and 44.4% (87/196) for Group 4 ($P < 0.01$). The interval (days) between first insemination and insemination before ultrasonography on Day 27 was 22.5 ($\pm 0.41$) days for cows in Group 1, 22.7 days ($\pm 0.47$) for cows in Group 2, 23.4 days ($\pm 0.39$) for cows in Group 3, and 21.5 days ($\pm 0.41$) for cows in Group 4 ($P = 0.05$).

Table 2
Pregnancy rates on Days 27 and 55 and pregnancy losses between Day 27 and 55 for cows in all groups

| Variable | Group 1 (GnRH Day 5) | | Group 2 (GnRH Day 15) | | Group 3 (GnRH Days 5 and 15) | | Group 4 (Control) | | $P$ |
|----------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|
|          | %  | N  | %  | N  | %  | N  | %  | N  |   |
| PR Day 27 | %  | %  | %  | %  | %  | %  | %  | %  |   |
| Pregnant  | 47.7  | 102/214 | 43.5  | 91/209 | 36.8  | 78/212 | 44.4  | 87/196 | 0.12 |
| Open      | 49.5  | 106/214 | 54.1  | 113/209 | 60.8  | 129/212 | 53.1  | 104/196 |   |
| QP        | 2.8  | 6/214  | 2.4  | 5/209  | 2.3  | 5/212  | 2.5  | 5/196  |   |
| PL        | %  | %  | %  | %  | %  | %  | %  | %  |   |
| Yes       | 19.6  | 20/102 | 13.2  | 12/91 | 23.0  | 18/78 | 19.5  | 17/87 | 0.40 |
| No        | 80.4  | 82/102 | 86.8  | 79/91 | 77.0  | 60/78 | 80.5  | 70/87 |   |
| PR Day 55 | %  | %  | %  | %  | %  | %  | %  | %  |   |
| Pregnant  | 39.3  | 84/214 | 38.8  | 81/209 | 28.3  | 60/212 | 36.2  | 71/196 | 0.06 |
| Open      | 60.7  | 130/214 | 61.2  | 128/209 | 71.7  | 152/212 | 63.8  | 125/196 |   |

PR: questionable pregnant. On Day 27, 2.5% (21/831) of cows were diagnosed as questionable pregnant. On Day 55, 76.2% (16/21) of the cows classified as questionable pregnant were determined to be not pregnant, and 23.8% (5/21) were confirmed pregnant (2 in Group 1, 2 in Group 2, and one in Group 4).

all groups adjusting for season are shown in Table 5. There was no difference in the risk of pregnancy loss between Days 27 and 55 among groups.

Cows in Group 3 (GnRH treatment on both Day 5 and Day 15) had a lower proportion of cows diagnosed not pregnant based on expression of estrus and insemination before ultrasonography on Day 27, and these cows had an extended interestrus interval compared with cows in Group 4 (untreated controls). The proportion of cows diagnosed not pregnant based on expression of estrus and insemination before ultrasonography on Day 27 was 46.2% (49/106) for Group 1, 42.3% (48/113) for Group 2, 25.6% (33/129) for Group 3, and 52.9% (55/104) for Group 4 ($P < 0.01$). The interval (days) between first insemination and insemination before ultrasonography on Day 27 was 22.5 ($\pm 0.41$) days for cows in Group 1, 22.7 days ($\pm 0.47$) for cows in Group 2, 23.4 days ($\pm 0.39$) for cows in Group 3, and 21.5 days ($\pm 0.41$) for cows in Group 4 ($P = 0.05$).

Table 3
Pregnancy rates on Day 27, and the AOR, 95% CI, and level of significance for the risk of nonpregnancy in cows in all groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pregnancy %</th>
<th>N</th>
<th>Risk of nonpregnancy AOR</th>
<th>95% CI</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 (GnRH Day 5)</td>
<td>47.7</td>
<td>102/214</td>
<td>0.9</td>
<td>0.6–1.3</td>
<td>0.11</td>
</tr>
<tr>
<td>2 (GnRH Day 15)</td>
<td>43.5</td>
<td>91/209</td>
<td>1.1</td>
<td>0.7–1.6</td>
<td>0.92</td>
</tr>
<tr>
<td>3 (GnRH Days 5 and 15)</td>
<td>36.8</td>
<td>78/212</td>
<td>1.4</td>
<td>0.9–2.1</td>
<td>0.03</td>
</tr>
<tr>
<td>4 (Control)</td>
<td>44.4</td>
<td>87/196</td>
<td>Referent</td>
<td>Referent</td>
<td>NA</td>
</tr>
<tr>
<td>Season</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>November–February</td>
<td>50.4</td>
<td>215/427</td>
<td>Referent</td>
<td>Referent</td>
<td>NA</td>
</tr>
<tr>
<td>March/April</td>
<td>37.3</td>
<td>143/383</td>
<td>1.7</td>
<td>1.3–2.2</td>
<td>0.0002</td>
</tr>
</tbody>
</table>

NA: not applicable.
4. Discussion

In the present experiment, GnRH administration on either Day 5 or Day 15 did not increase pregnancy rate on Day 27 or Day 55, and did not reduce pregnancy loss between Day 27 and Day 55. Moreover, administration of GnRH on both Day 5 and Day 15 reduced pregnancy rate on Day 27 and Day 55.

Previous research showed that administration of GnRH, GnRH agonist or hCG after insemination at detected estrus and at specific times coincident with the presence of the dominant follicle/s of the first and second follicular waves may stimulate CL function, induce accessory CL formation, increase P₄ and reduce estrogen production with a consequent positive effect on embryo survival [39].

Human chorionic gonadotropin administered on Day 7 after insemination induced accessory CL, increased P₄ concentration and reduced embryo mortality compared to cows treated on Day 0, Day 14, or nontreated controls [28]. A treatment with a GnRH analog (buserelin, 8 μg) on Day 7 after estrus in postpartum beef cows increased the number of
large luteal cells and size of the CL in cyclic cows, increased the number of large luteal cells of the buserelin-induced CL, and P₄ concentrations in noncyclic cows [40]. Treatment with hCG on Day 5 in dairy heifers [30,41] and in lactating dairy cows [42] or on Day 6 in beef cows [29] induced accessory CL formation and increased P₄ concentrations. Induction of accessory CL was similar, but the increase in P₄ concentration was higher in dairy heifers receiving hCG on Day 5 of the estrous cycle compared with a GnRH analog (buserelin, 8 μg; [30]). In addition, administration of hCG on Day 5 in lactating dairy cows increased pregnancy rate [42]. Similarly, insertion of an intravaginal progesterone device (CIDR-B) 6–8 days but not after mid-cycle postinsemination increased pregnancy rate to first insemination [43].

Administration of a GnRH agonist (fertirelin acetate 100 μg) between Day 11 and Day 14 after insemination increased P₄ concentration 4–12 days later and reduced estrogen concentrations on Days 8–12 in association with enhanced luteal function [44], and increased pregnancy rate in beef heifers [45]. A meta-analysis indicated an increase in pregnancy rate for beef and dairy cows receiving GnRH between Day 11 and Day 14 [46]. In cows subjected to TAI using the Ovsynch protocol, a treatment with GnRH (100 μg gonadorelin acetate) on Days 7, 14, or 7 and 14 increased plasma P₄ concentrations, but tended to increase pregnancy rate only in cows that responded to the synchronization protocol [47]. Administration of GnRH (100 μg gonadorelin diacetate) on Day 11 after TAI in lactating dairy cows subjected to the Ovsynch protocol during a period of mild heat stress increased plasma P₄ concentrations and pregnancy rate [48].

In the present study, treatment with GnRH either on Day 5 or Day 15 in lactating dairy cows subjected to synchronization of ovulation and TAI did not increase pregnancy rate on Days 27 and 55, and did not reduce the rate of pregnancy loss between Day 27 and Day 55. The lack of effect of GnRH either on Day 5 or Day 15 to increase pregnancy rate or reduce pregnancy loss is in agreement with other studies of cows inseminated at detected estrus and treated with either hCG or GnRH at specific times. Administration of hCG on Day 3 [49], or Day 4 [50] in beef heifers, on Day 4 in lactating dairy cows [51] or on Day 5 in dairy heifers [30] failed to increase pregnancy rate. Buserelin acetate administered either on Days 11 to 14 [52], Day 12 [51] or Day 15 or hCG on Days 10 or 15 [53] failed to increase pregnancy rate in lactating dairy cows.

The increase in pregnancy rates on Day 27 and Day 55 for cows receiving a dose of GnRH on Day 5 (P < 0.11 and <0.19) and the reduction in pregnancy losses (PL) between Day 27 and Day 55 for cows receiving a dose of GnRH on Day 15 (P < 0.11) were not statistically significant. A previous study showed an increase in pregnancy rate in lactating dairy cows treated with hCG on Day 5 after insemination at detected estrus [42]. In dairy heifers, hCG administered on Day 5 after detected estrus was more efficient than GnRH on inducing accessory CL and increasing P₄ concentration [30]. Perhaps, an increase in pregnancy rate or reduction in pregnancy losses in cows subjected to synchronization of ovulation and timed insemination could have been obtained by administering hCG at either Day 5 or Day 15.

Treatment with GnRH on both Day 5 and Day 15 reduced the proportion of cows diagnosed not pregnant based on expression of estrus and insemination before ultrasonography on Day 27, and extended the interval between the initial insemination (TAI) and the
next insemination in the present study. This is in agreement with previous research using buserelin daily from Day 9 to Day 12 (10 µg [54]), or between Day 11 and Day 13 (5 µg [55]; 10 µg [56]) which extended the estrous cycle in lactating dairy cows. However, in the present study, treatment with GnRH on both Day 5 and Day 15 reduced pregnancy rate on both Day 27 and Day 55.

There are several potential mechanisms which may explain the apparent detrimental effect of GnRH treatment on both Day 5 and Day 15 on pregnancy rate observed on Day 27 and Day 55. It has been shown that GnRH treatment of heifers on Day 2 or Day 10 of the estrous cycle reduced P₄ concentrations on Days 12, 14, and 16, and the number of unoccupied LH receptors in plasma membrane of the CL [57]. The majority of LH receptors are expressed in the small luteal cells [58], and small luteal cells are transformed into large luteal cells during CL development [59]. Therefore, in the present study, treatment of cows with GnRH on both Day 5 and Day 15 (Group 3) could have resulted in excessive LH stimulation which may have caused a premature switch from small luteal to large luteal cells, and a reduction of LH receptors resulting in a decrease in the luteotrophic effect of LH.

It has recently been reported that increased progesterone concentrations reduced pregnancy rate in cows treated with eCG after insemination [60]. In the present study, cows treated with GnRH on both Day 5 and Day 15 had an extended interluteal interval. This was the interval between the initial insemination (TAI) and the one at which they were inseminated prior to Day 27. This suggests an increased secretion of progesterone due to formation of accessory CL and/or induced turnover of a potential dominant follicle. However, these assumptions cannot be substantiated since the presence of an accessory CL, progesterone concentrations, and follicular dynamics were not determined for cows in this study.

From the results of this study, it was concluded that: (1) GnRH treatment on Day 5 or Day 15 did not increase pregnancy rate, or reduce pregnancy loss between Day 27 and Day 55 after TAI; (2) Cows treated with GnRH on both Day 5 and Day 15 had a lower proportion of cows diagnosed not pregnant based on expression of estrus before ultrasonography on Day 27 (26.5%) compared to control cows (52.9%), and these cows had an extended interluteal interval (23.4 days vs. 21.5 days); and (3) GnRH treatment on both Day 5 and Day 15 after TAI reduced pregnancy rate on Day 27 and Day 55 (36.8 and 28.3%) compared with control cows (44.4% and 36.2%). Therefore, strategies to stimulate CL function, induce ovulation of dominant follicles and accessory CL formation using multiple doses of GnRH during the luteal phase to enhance embryo survival in lactating dairy cows subjected to induction of ovulation need to consider the potential negative effects demonstrated in this study.

Acknowledgments

This study was funded by North Florida Holsteins, Inc. (NFH), Florida Dairy Check-Off, and USDA Intramural Funds from CVM, University of Florida. The authors thank Mr. Don Bennink for use of the dairy herd, and the entire staff of NFH for assistance with this study.
References


