Effect of GnRH administration at day 5 or day 7 after AI on progesterone concentrations, corpus luteum volume and pregnancy in lactating dairy cows

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Abstract

The objective of the study was to determine the effect of gonadotropin releasing hormone (GnRH) administration on day 5 or day 7 after artificial insemination (AI) on progesterone concentration, total corpus luteum (CL) volume and pregnancy/AI (P/AI) in lactating dairy cows. Multiparous dairy cows (n=1054) from two dairy farms, one in Virginia and one in North Carolina, were enrolled in this study. All cows were synchronized with Presynch-CO-Synch protocol for first service. Following AI (day 0), cows were randomly allocated to one of the following three treatment groups: cows in the GnRH-5 group (n=335) received 100 µg of GnRH im five days after insemination; cows in the GnRH-7 group (n=390) received 100 µg of GnRH im seven days after insemination and cows in the control group (n=329) received no treatment. Blood samples were collected from a subset of cows (n=52) at day -38, day -24, day -10, day 0 (day of AI) and day 14 for progesterone concentration determination. The progesterone concentrations at day -38, -28, and -10 determined ovarian cyclicity of the cows and the progesterone concentration at day14 (9 or 7 days following GnRH treatment) were used to detect differences in progesterone among treatment groups. The ovaries of the subset of cows were screened using ultrasonography for the formation of accessory CL and total volume of CLs (primary + accessory CLs) was recorded. Cows were examined for pregnancy by transrectal ultrasonography at 30 days and again at 60 days after insemination. The P/AI for GnRH-5, GnRH-7 and control groups were 31.6% (106/335), 33.8% (132/390) and 31.9% (105/329), respectively ($p > 0.1$). The total CL volume differed among the treatment groups ($p < 0.05$) but day 14 progesterone concentration did not differ among the treatment groups ($p > 0.1$). The progesterone concentration in the control group (n=17) was 5.9 ± 0.3 ng/ mL and total CL volume was 8.3 ± 0.6 cm^3. The progesterone concentration in the GnRH-5 group (n=15) was 6.3 ± 0.4 ng/ mL and total CL volume was 12.6 ± 0.7 cm^3. The progesterone concentration in the GnRH-7 group (n=20) was 6.6 ± 0.4 ng/ mL and total CL volume was 12.0 ± 0.8 cm^3. In conclusion, GnRH administration at day 5 or day 7 following insemination neither increased progesterone concentrations nor improved P/AI compared to controls.

Keywords: GnRH, progesterone, pregnancy rate, timed artificial insemination, dairy cattle, fertility

Introduction

Establishment and maintenance of pregnancy are complex processes that require precise communication between conceptus and dam. The role of progesterone is critical in these processes and insufficient luteal activity has been associated with infertility in cattle. Conception failure is coincident with less than normal concentrations of progesterone as early as six days after AI.[1] Blood progesterone generally reaches greater concentrations earlier in pregnant than in non-pregnant cows suggesting that strategies to increase progesterone concentration earlier following insemination may be beneficial in reducing embryo loss. Embryo development is related to concentrations of progesterone and the ability of the conceptus to secrete interferon-τ.[2] Mann et al tested the effect of timing of progesterone supplementation on embryo development and interferon-τ production in cows and concluded that both early (5 to 9 days) and later (12 to 16 days) progesterone supplementation resulted in significant increases in plasma progesterone. In addition they have found early progesterone supplementation resulted in a fourfold increase in trophoblast length ($p < 0.01$) and a six-fold increase in uterine concentration of interferon-τ ($p < 0.05$).[3] Hence more exposure of an embryo to progesterone may increase its chances of secreting interferon-τ and thus survive. Exogenous progesterone can stimulate embryo development, so by manipulating post-insemination concentrations of progesterone, conception rates may ultimately improve.[4] Several strategies have been used to increase progesterone concentration after breeding.[5] Administration of GnRH and human chorionic gonadotropin (hCG) during the early luteal phase induces

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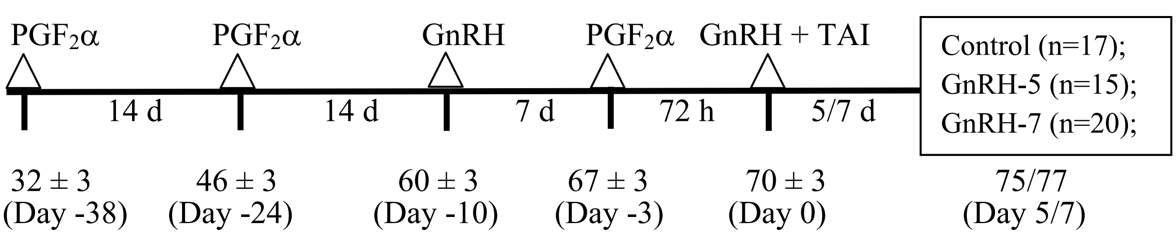
ovulation of the dominant follicle from the first follicular wave and results in formation of an accessory CL.[6] Injection of GnRH or its agonists induced accessory CL when administered on days 5 to 12 of the estrous cycle.[7,8] Differences in response to GnRH administration from days 5 to 12 of the estrous cycle exist and may be due to the difference in the size of the follicle present at that time. The follicle size on day 5 of the estrous cycle is smaller in cows with a longer inter-wave interval (two follicular waves) and renders them non-responsive to GnRH when administered at day 5. However these follicles may responsive to GnRH on day 7. The objective of the study was to determine the effect of GnRH administration on day 5 or day 7 after AI on progesterone concentration, total CL volume and P/AI in lactating dairy cows.

Materials and methods

Multiparous dairy cows (n=1054) from two dairy farms (one in Virginia and one in North Carolina) were enrolled in this study. All cows were synchronized with Presynch-CO-Synch protocol for first service (Figure). Briefly, cows received two injections of 25 mg prostaglandin $F_2\alpha$ (PGF) im (Lutalyse®, Pfizer Animal Health, New York, NY) at 32 ± 3 days postpartum and 46 ± 3 days postpartum (Presynch); 100 µg GnRH im (Cystorelin®, Merial Ltd. Duluth, GA) at 60 ± 3 days postpartum; 25 mg PGF at 67 ± 3 days postpartum; and 100 µg GnRH + timed AI (TAI) at 70 ± 3 days postpartum (CO-Synch; day 0). Body condition scores (1 to 5 scale; 1-emaciated; 5-obese) of all cows were recorded at the time of AI. Following AI, cows were blocked by body condition score and parity, and were randomly allocated to one of the following three treatment groups: cows in the GnRH-5 group (n=335) received 100 µg of GnRH im on day 5 after insemination; cows in the GnRH-7 group (n=390) received 100 µg of GnRH im on day 7 after insemination and cows in the control group (n=329) received no treatment. Blood samples were collected from a subset of cows (n=52) at 32, 46, 60, 70, and 84 days in milk (DIM) for progesterone concentration determination and the ovaries were screened using ultrasonography. Total volume of CLs (primary and accessory CL) was recorded. The CL volume was calculated using the formula $4/3\pi r^3$. The progesterone concentration at 60 DIM (at first GnRH the initiation of the Ovsynch protocol) determined ovarian cyclicity of the cows. Progesterone concentrations <1 ng/mL indicated a non-cycling cow. The progesterone concentration at 84 DIM (14 days after insemination) was used to measure differences among treatment groups in progesterone concentrations following GnRH administration (7 or 9 days after GnRH injection). Cows were examined for pregnancy by transrectal ultrasonography (Sonosite 180 plus, Sonosite Inc., Bothell, WA) at 30 days and again at 60 days after insemination.

Figure.



Progesterone assay

Blood samples were collected via coccygeal venipuncture into blood collection tubes (Vacutainer®, Becton Dickinson, Franklin Lakes, NJ), placed immediately on ice. The samples were brought to the laboratory and centrifuged at 3,000 x *g* for 15 minutes. Harvested serum was stored at -20°C until further processing. Progesterone concentration was determined using a Coat-A-Count kit (DPC Diagnostic Products Inc., Los Angeles, CA) solid-phase ^{125}I radioimmunoassay. The intra-assay and inter-assay coefficients of variation were 6.34% and 7.29%, respectively. The assay sensitivity was 0.03 ng/mL.

Statistical analyses

Data were analyzed by PROC LOGISTICS procedure of SAS (SAS version 9.12, SAS Institute Inc., Cary, NC) to determine the difference in P/AI among treatment groups. The variables included in the model were parity, season, farm, treatment x parity, treatment x season, and treatment x farm interactions. GLM procedure was used to determine the difference in progesterone and total CL volume among the treatment groups. Progesterone and total CL volume were tested for normality using Kolmogorov-Smirnov test and observed to be normally distributed. $P \leq 0.05$ was considered as significant.

Results

The P/AI for GnRH-5, GnRH-7 and control groups were 31.6% (106/335), 33.8% (132/390) and 31.9% (105/329), respectively (Table; $p > 0.1$). The P/AI was not different for parity, season, farms, treatment x parity, treatment x season, or treatment x farm interactions ($p > 0.1$).

The mean (±SD) CL volume differed among the treatment groups (Table; $p < 0.05$) but mean (±SD) progesterone concentration did not differ among the treatment groups ($p > 0.1$). The progesterone concentration for the control group (n=17) was 5.9 ± 0.3 ng/ mL and mean (±SD) total CL volume was 8.3±0.6 cm^3. The progesterone concentration for the GnRH-5 group (n=15) was 6.3 ± 0.4 ng/ mL and total CL volume was 12.6±0.7 cm^3. In the GnRH-5 group six cows failed to develop accessory CL. In the GnRH-5 group, the progesterone concentration and total CL volume for the cows that did not develop accessory CL were 4.7 ± 0.3 ng/ mL and 9.2±0.5 cm^3, respectively, and for the cows that did develop accessory CL were 7.4 ± 0.3 ng/ mL and 14.8±0.7 cm^3. The progesterone concentration for the GnRH-7 group (n=20) was 6.6 ± 0.4 ng/ mL and total CL volume was 12.0±0.8 cm^3. In the GnRH-7 group ten animals failed to develop accessory CL. In the GnRH-7 group, the progesterone concentration and total CL volume for the cows that did not develop accessory CL were 6.6 ± 0.5 ηg/ mL and 7.7±0.8 cm^3, respectively, and for the cows that did develop accessory CL were 6.5 ± 0.4 ηg/ mL and 16.2±0.9 cm^3. The P/AI for the control, GnRH-5 and GnRH-7 groups at 30 days after insemination in the subset of cows was 76.5% (13/17), 80.0 % (12/15) and 65.0% (13/20), respectively. The P/AI in the subset of cows for the control, GnRH-5 and GnRH-7 groups at 60 days after insemination was 70.6% (12/17), 73.3 % (11/15) and 50.0% (10/20), respectively. One cow with accessory CL in GnRH-5 group and three cows with no accessory CL in the GnRH-7 group lost their pregnancies between 30 and 60 days of gestation.

Of the 52 cows, ovarian cyclicity affected the P/AI [cycling: 54.5% (43/79); non-cycling 15.6% (5/32); $p < 0.02$]. There was no effect of treatment or treatment x ovarian cyclicity on P/AI ($p > 0.1$).

Discussion

Treatment with GnRH on day 5 or day 7 following insemination neither increased progesterone concentrations nor improved P/AI compared to controls.

Corpus luteum formation and development is highly responsive to LH, and small luteal cells synthesize more progesterone when stimulated by LH.[9] The administration of GnRH or LH during the early luteal phase induces ovulation of the first-wave dominant follicle and formation of a functional accessory CL. Schmitt et al observed that most of the increase of progesterone production after hCG injection was due to the formation of accessory CL.[10] Furthermore, cows treated with hCG on day 5 after estrus had a greater increase in plasma progesterone from day 6 to 13 and greater circulating progesterone on day 13 of the estrous cycle. However, Sterry et al observed that GnRH did not affect circulating progesterone concentrations from 5 to 19 days after TAI.[11] In this study, 40% and 50% of cows failed to develop accessory CL when GnRH was administered on day 5 and day 7, respectively. It should be noted that progesterone determination was from a subset of 52 cows only. Interestingly, in the GnRH-5 group, the progesterone concentration was higher in cows that developed accessory CL compared to cows that did not develop accessory CL; however this difference was not observed in the GnRH-7 group. In both the GnRH-5 and the GnRH-7 group the total CL volume was higher in cows that developed accessory CL compared to cows that did not. These observations (increased total CL volume and similar progesterone concentrations in cows with accessory CL compared to cows with no accessory CL) indicate that individual variation in dairy cow metabolism might play a role in modulating circulating progesterone concentrations. Higher producing cows generally consume more dry matter, and feed intake can affect plasma progesterone concentrations of dairy cows.[12,13]

Previous studies have found conflicting results on conception rates in cows receiving hCG.[14-16] Sianangama and Rajamahendran and Santos et al observed a remarkable increase in conception rate in cows receiving hCG on day 7 and day 5 after AI, respectively.[16,17] However, other authors report no differences in conception rate between control and hCG-treated cows.[14,15] In the latter studies, the conception rate of control cows (conception rate > 59%) was markedly similar to those in this study.[13,14] It is likely that such highly fertile groups of cows might not benefit from hCG treatment. In addition, a limited number of cows and different protocols and dosage regimens were utilized, which might have affected the ability to detect any difference between treatment groups. Sterry et al conducted experiments in which cows were assigned to either control (n = 223), intravaginal progesterone (n = 218), or GnRH 5 days after TAI (n = 227) treatments in one experiment and in another cows were assigned to control (n = 160), GnRH 5 days after TAI (n = 159), or GnRH 7 days after TAI (n = 163).[11] They observed that the treatment did not affect P/AI in these experiments; however, when data were combined to compare control animals (n = 383) with those treated with GnRH 5 days after TAI (n = 386), P/AI tended to be greater in those treated with GnRH 5 days after TAI (49.1%) than in control cows (45.8%). They also have observed that the effect resulted from a GnRH treatment x cyclicity status interaction in which P/AI for non-cycling cows receiving GnRH 5 days after TAI was greater than for noncycling control cows (45.5 vs. 31.1%). No treatment x cyclicity interactions observed in the current study.

Blood progesterone (timing and magnitude) generally reaches greater concentrations earlier in pregnant than in non-pregnant cows suggesting that strategies to increase progesterone concentration earlier following insemination may be beneficial in reducing embryo loss. Santos et al observed an increase in conception rate for cows receiving hCG on day 5 after AI.[17] Ginther et al indicated that deviation of the dominant follicle takes place two to three days after a new cohort of follicles is recruited.[15] The dominant follicle acquires LH receptors between two and four days after wave emergence when it reaches 8 to 10 mm in diameter.[18,19] Therefore, on day 5 of the estrous cycle, dairy cows should have a dominant follicle that is responsive to LH and is capable of ovulating when ovulation is induced by hCG. It is reasonable to expect a similar response when GnRH is administered on day 5 or day 7. However, it is possible that the increased response to hCG administration on day 5 is due to its longer half-life compared to GnRH.

In conclusion, GnRH administration on day 5 or day 7 following insemination neither increased progesterone concentrations nor improved P/AI.

Table. Effect of treatment, parity, season and farms on the odds of pregnancy in lactating dairy cows

Predictor	Coefficient ± SE	P	Odds Ratio	95% Lower	95% Upper
Constant	-0.6265 ± 0.3451	0.069			
Treatment[†]	0.1119 ± 0.2542	0.66	1.12	0.68	1.84
Parity[‡]	-0.1481 ± 0.1073	0.168	0.86	0.7	1.06
Season[§]	0.0612 ± 0.0932	0.511	1.06	0.89	1.28
Farm[**]	-0.1894 ± 0.1431	0.462	0.89	0.71	1.05
Treatment x Parity	0.0176 ± 0.7945	0.824	1.02	0.87	1.19
Treatment x Season	-0.0440 ± 0.0679	0.517	0.96	0.84	1.09
Treatment x Farm	0.0248 ± 0.0574	0.852	0.98	0.81	1.10

[†]Treatment: GnRH administration on day 5 or day 7 or control;
[‡]Parity Categories: 1, 2 or ≥3;
[§]Season: Fall, Winter, Spring or Summer;
[**]2 farms

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