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Ovarian responses and conception rates in response to gNrh, Hcg, and progesterone

Abstract

We hypothesized that increasing concentrations of progesterone after artificial insemination (AI) would increase fertility. Our objective was to assess changes in ovarian structures, incidence of ovulation, and change in serum progesterone in response to GnRH, human chorionic gonadotropin (hCG), or exogenous progesterone (controlled internal drug release; CIDR insert) treatment, beginning 4 to 9 days after AI (d 0) and again 7 days later (Exp. 1). Blood was collected from 753 cows in 3 herds on days 0 and 7. Ovaries of 162 cows in 1 herd were scanned and mapped to confirm the presence a corpus luteum (CL), and cows were assigned randomly to serve as control (n = 41) or to receive a CIDR insert for 7 days (n = 41), 100 µg of GnRH (n = 40), or 3,300 IU of hCG (n = 40). More cows were induced to ovulate in response to GnRH (60%) and hCG (78%), compared with control (2.4%). Compared with control, cows treated with GnRH or hCG had more induced CL (d 7) and more total CL (d 7), but serum progesterone was increased only in response to hCG. Volume of the original luteal structures was increased by hCG, but tended to be reduced by CIDR and GnRH, compared with luteal volume in control. Total CL volume was increased by hCG, but reduced by CIDR, compared with CL volume of control. In Exp. 2, cows in 5 herds were used to assess conception rates in response to the same treatments described in Exp. 1: control (n = 708), CIDR (n = 711), GnRH (n = 719), and hCG (n = 714). Tendencies for interactions of treatment x herd and treatment x lactation group were detected, but no 3-way interactions were found. Treatment with hCG increased conception rates in second-lactation cows. The CIDR tended to increase, and hCG increased, conception rates in 2 herds, whereas the CIDR decreased conception rates in 1 herd. We concluded that GnRH and hCG effectively induced ovulation, and increased number of CL, but only hCG increased serum progesterone. Further, treatment with the CIDR or hCG increased conception rates, but only in some herds.; Dairy Day, 2006, Kansas State University, Manhattan, KS, 2006;

Keywords

Diary Day, 2006; Kansas Agricultural Experiment Station contribution; no. 07-118-S; Report of progress (Kansas State University. Agricultural Experiment Station and Cooperative Extension Service); 965; Dairy; CIDR; GnRH; hCG; Pregnancy rate

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**OVARIAN RESPONSES AND CONCEPTION RATES
IN RESPONSE TO GnRH, hCG, AND PROGESTERONE¹**

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Summary

We hypothesized that increasing concentrations of progesterone after artificial insemination (AI) would increase fertility. Our objective was to assess changes in ovarian structures, incidence of ovulation, and change in serum progesterone in response to GnRH, human chorionic gonadotropin (hCG), or exogenous progesterone (controlled internal drug release; CIDR insert) treatment, beginning 4 to 9 days after AI (d 0) and again 7 days later (Exp. 1). Blood was collected from 753 cows in 3 herds on days 0 and 7. Ovaries of 162 cows in 1 herd were scanned and mapped to confirm the presence a corpus luteum (CL), and cows were assigned randomly to serve as control (n = 41) or to receive a CIDR insert for 7 days (n = 41), 100 µg of GnRH (n = 40), or 3,300 IU of hCG (n = 40). More cows were induced to ovulate in response to GnRH (60%) and hCG (78%), compared with control (2.4%). Compared with control, cows treated with GnRH or hCG had more induced CL (d 7) and more total CL (d 7), but serum progesterone was increased only in response to hCG. Volume of the original

luteal structures was increased by hCG, but tended to be reduced by CIDR and GnRH, compared with luteal volume in control. Total CL volume was increased by hCG, but reduced by CIDR, compared with CL volume of control. In Exp. 2, cows in 5 herds were used to assess conception rates in response to the same treatments described in Exp. 1: control (n = 708), CIDR (n = 711), GnRH (n = 719), and hCG (n = 714). Tendencies for interactions of treatment × herd and treatment × lactation group were detected, but no 3-way interactions were found. Treatment with hCG increased conception rates in second-lactation cows. The CIDR tended to increase, and hCG increased, conception rates in 2 herds, whereas the CIDR decreased conception rates in 1 herd. We concluded that GnRH and hCG effectively induced ovulation, and increased number of CL, but only hCG increased serum progesterone. Further, treatment with the CIDR or hCG increased conception rates, but only in some herds.

(Key Words: CIDR, GnRH, hCG, Pregnancy Rate.)

¹Select Sires, Waupun, WI, and Plain City, OH, respectively.

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Introduction

Conception failure is coincident with less-than-normal concentrations of progesterone as early as day 6 after insemination. In general, blood concentrations of progesterone rise earlier and achieve greater concentrations in pregnant, than in nonpregnant, cows. Embryo development is associated with concentrations of progesterone and the ability of the conceptus to secrete the antiluteolytic hormone, interferon-tau. Exogenous progesterone has been shown to stimulate embryo development. A number of treatments could be employed to increase peripheral concentrations of progesterone after AI, including those that increase endogenous function of the existing corpus luteum (CL), induce accessory CL, or supplement progestin or progesterone directly.

Human chorionic gonadotropin (hCG) has activity similar to LH, is able to bind to tissue LH receptors, and mimics effects of LH by causing small luteal cells to increase progesterone synthesis. Administration of hCG increases the incidence of ovulation and accessory CL formation. In addition, luteal phase treatment with hCG after AI increased conception rates in some studies.

Inducing accessory CL with GnRH or its agonists is well documented and forms the basis for the first GnRH injection of the Ovsynch protocol. Other research has demonstrated that incidence of ovulation was greatest when GnRH was injected between days 5 and 12 of the estrous cycle.

Studies that administered exogenous progestins during the luteal phase after AI by applying a progesterone-releasing intravaginal device (PRID) for 7 days produced inconsistent effects on conception rates. No increase in conception rates was detected when intravaginal controlled internal drug

release (CIDR) inserts were applied mid-cycle or later. In contrast, when treatments were initiated before midcycle, conception rates were improved for cows treated with CIDR inserts for 6 or 12 days, beginning 4 to 9 days after AI, compared with control (Macmillan and Peterson, 1993). The treatment that most consistently improved conception rates in that study was a 6- or 12-d CIDR insert beginning on 6 to 8 days after AI.

We hypothesized that increasing or supplementing endogenous concentrations of progesterone in lactating dairy cattle early after AI may spare embryonic loss and improve overall conception rates. Our overall objective was to investigate the effect of supplemental blood progesterone and exogenous GnRH and hCG on follicular development, incidence of ovulation, serum progesterone, and conception rate.

Procedures

Lactating Holstein cows were blocked by days in milk and lactation number (1 vs. 2+) and assigned randomly to 1 of 4 treatments: 1) insert a new CIDR (Eazi-Breed CIDR insert containing 1.38 g of progesterone; Pfizer Animal Health, New York, NY) for 7 days, beginning between 4 and 9 days after AI; 2) 3,300 IU of hCG, i.m. (Chorulon; Intervet, Millsboro, NJ) once between 4 and 9 days after AI; 3) 100 µg of GnRH, i.m. (Fertagyl; Intervet) once between days 4 and 9 after AI; and 4) untreated control. Body condition scores (1 = thin and 5 = fat) were assigned at treatment.

Experiment 1. The purpose of Exp. 1 was to assess ovarian responses to GnRH, hCG, and exogenous progesterone (CIDR insert). Ovaries of 162 lactating Holstein cows housed at the Kansas State University Dairy Teaching and Research Center were scanned by using transrectal ultrasonography. Ovarian

structures were mapped and sized on the day of treatment and 7 days later to determine the incidence of ovulation in response to treatment.

Blood was collected at the initiation of treatments and 7 days later to measure treatment effects on serum concentrations of progesterone. Cows located in the Kansas State University dairy, and at 2 commercial dairy farms in which Exp. 2 was conducted, had blood samples collected as described relative to treatments. Blood serum concentrations of progesterone were determined by radioimmunoassay.

Experiment 2. The purpose of Exp. 2 was to assess effects of treatments on conception. A total of 2,852 lactating Holstein cows were treated in the Kansas State University herd and at 4 commercial dairy locations (3 herds in Kansas and 1 herd in Wisconsin). Pregnancy was diagnosed by using either transrectal ultrasonography or transrectal palpation, and pregnancy was reconfirmed in all pregnant cows at 14 to 30 days. Conception rates were calculated based on the number of pregnant cows at each diagnosis divided by the number of cows previously inseminated and treated.

Results and Discussion

Retention of CIDR inserts during 7 days was 94.5% of 752 cows treated with CIDR inserts. Only 711 cows in which the CIDR was retained according to protocol were included in analyses. Cows were treated after AI, which included those occurring at 1 fixed time (timed AI; TAI), after visual detection of estrus, or in response to rubbed tail chalk, activity tags, and other miscellaneous signs of estrus. All cows were inseminated postpartum (first AI after calving) after presynchronization of estrous cycles (Presynch) and synchronization of ovulation (Ovsynch). In cows diagnosed not pregnant,

ovulation was resynchronized by using Ovsynch. More than 80% of inseminations that preceded treatments in both experiments were by TAI.

Experiment 1. Ovarian characteristics of 162 treated cows are summarized in Table 1. Because a large percentage of the cows eligible for treatment at 4 to 9 days after AI were previously TAI, only cows that had a CL consistent in size for that stage of the estrous cycle or pregnancy at the first ultrasound exam were included in Exp. 1. Mean diameter, just before treatment, of follicles that eventually ovulated in response to either GnRH or hCG did not differ (13.8 ± 0.5 vs. 12.7 ± 0.4 mm). More ($P < 0.01$) cows treated with GnRH and hCG ovulated at least 1 follicle than did control cows. When only cows having at least 1 follicle ≥ 10 mm in diameter at treatment were considered, percentage of follicles that ovulated increased to 64.1% and 81.3% for GnRH and hCG cows, respectively (Table 1). Number of induced CL per cow and total CL per cow 7 days after treatment were greater ($P < 0.01$) in cows treated with GnRH and hCG than in control cows (Table 1).

As expected, original CL increased in diameter and volume during 7 days after treatment in all cows, but increases in diameter ($P = 0.07$) and volume ($P < 0.05$) tended to be, or were, greater for cows treated with hCG than for control cows. In contrast, change in volume of original CL after 7 days in cows from CIDR and GnRH treatments tended ($P = 0.07$) to be less than in control cows. As a consequence, total luteal volume 7 days after treatment was less ($P < 0.01$) in CIDR treatment, but greater in hCG treatment, compared with control (Table 1).

Concentrations of progesterone in serum at the time of treatment and 7 days later were determined in 753 cows in 3 herds. As

expected, average concentration of progesterone in blood serum increased ($P < 0.001$) from day of treatment (4 to 9 days after AI; 3.2 ± 0.1 ng/mL) until 7 days later. Increase in serum progesterone from treatment to 7 days later, however, was greater ($P < 0.001$) in cows treated with hCG than in control cows (Figure 1).

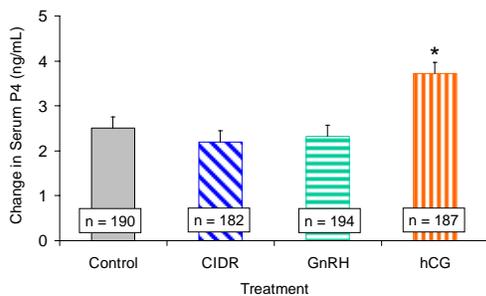


Figure 1. Change in Concentrations of Progesterone in Serum Between Day of Treatment and 7 Days Later for Cows in Exp. 1. *Different ($P < 0.001$) from control.

Experiment 2. Conception rates of 2,852 cows in 5 herds described in Tables 2 and 3 were assessed by ultrasonography in 2 herds (herds 1 and 3) and by palpation per rectum of uterine contents in 3 herds (herds 2, 4, and 5). Tendencies were detected for interactions of treatment \times herd ($P = 0.11$) and treatment \times lactation group ($P = 0.07$), but no 3-way interactions were detected. Treatment with the CIDR tended to ($P < 0.10$) increase, or increased ($P < 0.01$), conception rates in herds 1 and 3, whereas the CIDR decreased ($P < 0.05$) conception rate in herd 4, compared with rates in the control herd (Table 3). Treatment with hCG increased ($P < 0.05$) conception rates in herd 3. Treatment with

hCG increased ($P < 0.05$) conception rates in second-lactation cows (Table 4). Overall, a priori contrasts indicated that the CIDR tended ($P = 0.075$) to increase and hCG increased ($P < 0.05$) conception rates, compared with control (Table 3).

Conception rates were influenced by herd; rates were greater ($P < 0.05$) in 2 herds (herd 1 = 38.9% and herd 3 = 36.6%) in which pregnancy was diagnosed earlier after AI, compared with the 3 herds in which pregnancy was determined later by palpation (herd 2 = 30.9%, herd 4 = 29.3%, and herd 5 = 23.9%). Month of treatment ($P < 0.05$), days in milk ($P < 0.001$), body condition score ($P < 0.001$), and number of days after AI when treatment was initiated ($P < 0.05$) influenced conception rates, whereas most recent test-day milk yield had no effect. Average day effects across herds indicated that conception rates increased when treatment occurred after day 6 (day 4 = 26.6%, day 5 = 27.6%, day 6 = 26.2%, day 7 = 34.1%, day 8 = 32.5%, and day 9 = 44.5%; no treatment \times day interaction). Conception rates in response to treatments, based on day after AI when treatment was initiated, are illustrated in Figure 1.

Treatment of lactating dairy cows once with GnRH and hCG between 4 and 9 days after AI effectively induced ovulation, and increased number of CL (not total CL volume after GnRH), but only increased serum P4 in hCG-treated cows. Further, treatment with the CIDR tended to increase, and treatment with hCG increased, conception rates, but only in some herds. Treatment of second-lactation cows with hCG increased conception rates. Further work is warranted to determine how and when vaginally applied progesterone via the CIDR may influence conception rates.

Table 1. Ovarian Characteristics Before (day 0) and After (day 7) Post-insemination Treatments of Progesterone (CIDR insert), GnRH, and hCG in Lactating Dairy Cows (Exp. 1)

Trait	Treatment ¹			
	Control	CIDR	GnRH	hCG
Cows, no.	41	41	40	40
	Mean ± SE (n) or % (n)			
Induced ovulation				
% of cows	2.4 (41)	4.9 (41)	60.0** (40)	77.5** (40)
% of follicles	2.9 (34)	5.6 (36)	64.1** (25)	81.3** (37)
Induced CL, (day 7)	0.1 ± 0.1 (41)	0.1 ± 0.1 (41)	0.7 ± 0.1** (40)	1.1 ± 0.1** (40)
Total CL (day 7)	1.6 ± 0.1 (41)	1.3 ± 0.1 (41)	2.0 ± 0.1* (40)	2.4 ± 0.1** (40)
Change in diameter of original CL ² , mm	3.1 ± 0.7 (55)	2.4 ± 0.7 (50)	1.6 ± 0.7 (50)	4.7 ± 0.7† (52)
Change in volume of original CL ² , mm ³	3,131 ± 619 (55)	1,350 ± 662† (50)	1,446 ± 652† (50)	4,766 ± 640* (52)
Total luteal volume (day 7), mm ³	12,298 ± 1106 (41)	8,008 ± 1101** (41)	12,373 ± 1117 (41)	18,410 ± 1108** (41)

†Different ($P = 0.07$) from control.

*Different ($P < 0.05$) from control.

**Different ($P < 0.01$) from control.

¹Cows were treated once with GnRH, hCG, or a CIDR insert, beginning 4 to 9 days after AI. The CIDR insert was removed 7 days later.

²Trait assessed on day 7 minus that on day 0.

Table 2. Conception Rates by Herd in Response to Post-insemination Treatments of Progesterone (CIDR insert), GnRH, and hCG in Lactating Dairy Cows (Exp. 2)

Herd	Treatment ¹ , % (n)			
	Control	CIDR	GnRH	hCG
1	31.7 (41)	50.1† (40)	32.6 (40)	38.7 (38)
2	26.0 (158)	30.8 (158)	28.6 (159)	34.0 (158)
3	26.9 (143)	40.3** (162)	31.3 (153)	37.8* (153)
4	33.8 (206)	23.4* (204)	29.8 (209)	33.7 (209)
5	23.8 (160)	22.3 (147)	20.2 (158)	25.6 (156)
Total ²	28.3 (708)	32.7 (711)	28.1 (719)	33.6 (714)

†Different ($P < 0.10$) from control within herd, based on unadjusted Chi-square.

*Different ($P < 0.05$) from control within herd, based on unadjusted Chi-square.

**Different ($P < 0.01$) from control within herd, based on unadjusted Chi-square.

¹Cows were treated with once GnRH, hCG, or a CIDR insert, beginning 4 to 9 days after AI. The CIDR insert was removed 7 days later.

²A tendency ($P = 0.11$) for a treatment by herd interaction. Adjusted a priori contrasts: CIDR vs. control ($P = 0.075$) and hCG vs. control ($P < 0.05$).

Table 3. Conception Rates by Lactation Group in Response to Post-insemination Treatments of Progesterone (CIDR insert), GnRH, and hCG in Lactating Dairy Cows (Exp. 2)

Lactation Number	Treatment ¹ , % (n)			
	Control	CIDR	GnRH	hCG
First	32.8 (246)	34.9 (252)	36.0 (249)	33.2 (250)
Second	26.0 (204)	34.0 (203)	27.1 (208)	39.6**(208)
Third	26.5 (258)	31.2 (256)	22.3 (262)	29.1 (256)

**Different ($P < 0.01$) from control within lactation number, based on unadjusted Chi-square.

¹Cows were treated once with GnRH, hCG, or a CIDR insert, beginning 4 to 9 days after AI. The CIDR insert was removed 7 days later.