HUMAN CHORIONIC GONADOTROPIN AND GnRH EFFECTS ON PREGNANCY SURVIVAL IN PREGNANT COWS AND RESYNCHRONIZED PREGNANCY RATES

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Summary

Experiments have shown human chorionic gonadotropin (hCG) to be more effective than gondadotropin releasing hormone (GnRH) as a means to ovulate follicles. Experiment 1 determined the effects of replacing the first injection of GnRH (day 7) with hCG or saline in a Resynch-Ovsynch protocol on pregnancy rates in cows subsequently diagnosed not pregnant and pregnancy survival in cows subsequently diagnosed pregnant (day 0). A second study determined the ovulation potential of hCG compared with GnRH and saline (Exp. 2). In Exp. 1, cows in 4 herds were assigned randomly based on lactation number, number of previous AI, and last test-day milk yield to treatments of 1,000 IU of hCG, 100 µg of GnRH, or left as untreated controls 7 days before pregnancy diagnosis. Cows found not pregnant were given $PGF_{2\alpha}$ (day 0), then inseminated 72 hours later, concurrent with a GnRH injection (3 herds) or given GnRH 16 to 24 hours before AI at 72 hours (1 herd). Timed AI pregnancy rates tended ($P = 0.08$) to be reduced by saline (12.9%; n = 505) compared with GnRH (17.9%; n = 703) but not hCG (16.5%; n = 541). Among pregnant cows treated, pregnancy survival 4 to 9 weeks after initial pregnancy diagnosis differed among herds ($P < 0.001$); but in 1 herd, GnRH reduced pregnancy survival, whereas hCG seemed to increase survival compared with control. Only small differences were detected in the other 3 herds, except for a slight negative effect of hCG compared with control in 1 herd. Ovarian structures were monitored in herd 1 by using transrectal ultrasonography 0 and 7 days after treatment with hCG, GnRH, or saline (Exp. 2). A tendency for a treatment × pregnancy status interaction ($P = 0.07$) was detected. Incidences of ovulation in nonpregnant cows were: hCG (51.6%; n = 126), GnRH (46.1%; n = 102), and control (28.1%; n = 96), whereas those in pregnant cows were: hCG (59.3%; n = 59), GnRH (24.5%; n = 49), and saline (6.9%; n = 58). We concluded that: 1) initiating a Resynch-Ovsynch protocol 7 days before pregnancy diagnosis with saline reduced timed AI pregnancy rates (Exp. 1); 2) in pregnant cows treated with GnRH, pregnancy survival was slightly reduced in 1 of 4 herds (Exp. 1); and 3) incidence of new corpus luteum (CL) was greater after hCG than GnRH in pregnant cows but not in nonpregnant cows (Exp. 2).

(Key words: hCG, GnRH, pregnancy rates.)

Introduction

Ovulation synchronization protocols that facilitate fixed-time artificial insemination (TAI) have been a reality for several years. Many producers use these programs, with 77% of respondents to a recent survey resynchronizing repeat AI. Although these programs offer the opportunity to facilitate the use of TAI without detection of estrus, pregnancy rates historically have been compromised. About 10 to 30% of Ovsynch-treated cows failed to have synchronized ovulation. Although presynchronization treatments have shown effectiveness in increasing the number of females with a synchronized ovulation, they

are not suitable for use before resynchronization.

Most ovulation synchronization schemes use GnRH to control follicular development and induce ovulation of a dominant follicle. Research has shown that human chorionic gonadotropin (hCG) is more effective than GnRH at causing these follicles to ovulate. A minimum effective dose of hCG to induce ovulation, however, has not been documented.

We hypothesized that replacing the first injection of GnRH in a Resynch-Ovsynch protocol with hCG would induce more follicles to ovulate, subsequently improving synchronization and pregnancy rate at TAI. In addition, we hypothesized that the greater number of ancillary CL would increase progesterone concentrations in pregnant cows, thus reducing the incidence of pregnancy loss. Our overall objective was to develop an ovulation resynchronization protocol that increases the risk of conception, reduces the risk of pregnancy loss, and allows for TAI in dairy cattle.

Procedures

Experiment 1 was conducted at Kansas State University, as well as 3 commercial northeast Kansas locations. Experiment 2 was conducted at the Kansas State University Dairy Teaching and Research Center, Manhattan, KS. All research at Kansas State University was conducted from October 2005 until October 2006. Research at the 3 commercial locations was performed between March and November 2006.

The experimental design is presented in Figure 1. Seven days before pregnancy diagnosis, dairy cows, along with a few nulliparous dairy heifers (herd 1 only), were assigned randomly to treatments of hCG, GnRH, or saline. Treatments were assigned based on lactation number, number of previous AI, and last test-day milk yield. Pregnancy was diagnosed 1 week later (day 0).

Experiment 1

One week before pregnancy diagnosis, dairy cows at 4 Kansas dairies were assigned to receive 100 µg of GnRH (Fertagyl, Intervet Inc.), 1,000 IU of hCG (Chorulon, Intervet Inc.), or left as untreated controls based on lactation number, number of previous AI, and last test-day milk weight. Cows were diagnosed for pregnancy by transrectal ultrasonography on days 30 to 43 (herd 1) or by transrectal palpation on days 37 to 45 (herds 2 to 4) post-insemination. When cows (n = 1,235) were diagnosed pregnant, the resynchronization protocol was discontinued and pregnancy status was reassessed 4 to 9 weeks later to determine pregnancy survival.

Cows diagnosed not pregnant (n = 1,748) were given $PGF_{2\alpha}$ at diagnosis and received one TAI 72 hours later. Cows at 3 locations were administered 100 µg of GnRH (Fertagyl, Intervet Inc.) at the time of AI, whereas cows in herd 1 were given GnRH 16 to 24 hours before TAI. Following TAI, all cows not detected in estrus and inseminated were again diagnosed for pregnancy 30 to 45 days later. Some nonpregnant cows in the 3 commercial dairies were inseminated early based on activity, standing estrus, and chalk rubs. These cows were eliminated from the results and were not included in analyses.

Palpation pregnancy rate was calculated as the number of pregnant cows at each diagnosis divided by the number of cows presented for pregnancy diagnosis. Pregnancy rate was calculated as the number of pregnant cows at each diagnosis divided by the number of cows previously inseminated and treated. Pregnancy survival between the first and second pregnancy diagnosis (4 to 9 weeks later) also was examined.

Experiment 2

In herd 1, transrectal ultrasonography was conducted at the initiation of the resynchronization protocol before treatment. Ovarian structures were mapped, and follicles were sized. Structures were monitored again 7 days later, and new CL that were not present or visible at the first ultrasound were noted. Corpora lutea corresponding to large follicles at the first ultrasound were assumed to have ovulated in response to treatment.

Blood samples were collected from a coccygeal blood vessel at the time of each ultrasonography exam. Samples were stored on ice until transported to the laboratory for centrifugation. The serum portion was retained and frozen, and serum concentrations of progesterone were later quantified by radioimmunoassay.

Results and Discussion

Experiment 1

Herd palpation pregnancy rates for the 4 herds were: Herd 1 = 35.3% (n = 434), herd 2 = 35.3% (n = 881), herd 3 = 33.55 (n = 932) and herd 4 = 39.6% (n = 1,264). Herd 4 had greater ($P = 0.05$) palpation pregnancy rates than herds 2 and 3.

Pregnancy survival 4 to 9 weeks after initial pregnancy diagnosis by treatment based on postpartum insemination number is illustrated in Table 1 for 1,236 cows. Overall, no difference in pregnancy survival was detected between cows treated with hCG (93.6%; n = 420) 7 days before pregnancy diagnosis and those left as untreated controls (95.3%; n = 403). Pregnancy survival, however, tended ($P = 0.06$) to be reduced in those cows treated with GnRH (93.0%; n = 413) compared with controls. Herd ($P = 0.004$) and season ($P < 0.05$) affected pregnancy survival. Herd tended to have or had an effect on pregnancy survival at the first ($P = 0.10$), second ($P < $

0.01), and third ($P = 0.002$) inseminations post-AI. Herd 1, 2, 3, and 4 had survival rates of 85.1, 99.6, 91.2, and 94.2%, respectively. Neither lactation number nor last test-day milk weight had an effect on pregnancy survival. Lactation number however, tended ($P = 0.11$) to affect pregnancy survival for cows that conceived at their first postpartum insemination, with older cows having less survival (89%) than first-lactation cows (95%).

A treatment × herd interaction ($P = 0.004$) is illustrated in Figure 2. In herd 1, cows treated with hCG exhibited the greatest pregnancy survival, whereas survival was compromised in cows treated with GnRH compared with controls. Herd 3, however, exhibited reduced survival in females treated with hCG compared with those treated with GnRH and those left as untreated controls. Herds 2 and 4 responded similarly to treatment, with survival rates being comparable across all treatments.

Resynchronized timed AI pregnancy rates by treatment based on postpartum insemination number is illustrated in Table 2 for a total of 1,749 inseminations in 4 herds. Overall, no difference ($P = 0.17$) in pregnancy rate was detected between cows treated with hCG (16.5%; n = 541) and those treated with GnRH (17.9%; n = 703) or left as untreated controls (12.9%; n = 505) 7 days before pregnancy diagnosis. Pregnancy rate for GnRH-treated cows, however, tended ($P = 0.08$) to differ from that of controls. Treatment, herd, lactation, and treatment × lactation interaction had no effect on the risk of pregnancy. In contrast, for each 10kg increase in last test-day milk weight, pregnancy rate decreased (P < 0.05) by 2.2 ± 1%. Sire nested within herd ($P = 0.007$) and season nested within herd ($P = 0.018$) had an effect on pregnancy rate, whereas technician nested within herd did not ($P = 0.79$).

Figure 3 illustrates a treatment × herd interaction ($P < 0.05$) on timed AI pregnancy rate. Herds 1 and 4 responded similarly to treatment as did herds 2 and 3. Cows treated with hCG and those treated with GnRH had the greatest pregnancy rates numerically in 2 herds each. Untreated controls seemed to have reduced pregnancy rates in 1 herd, but fertility was comparable with at least 1 treatment in the other 3 herds.

Experiment 2

Ovaries of 490 cows were monitored for ovulation 7 days after treatment (pregnancy diagnosis) with hCG, GnRH, or saline. Treatment affected ($P < 0.001$) incidence of ovulation with 52.4% (n = 185), 39.1% (n = 151), and 20.1% (n = 154) of hCG, GnRH, and saline treated cows ovulating, respectively. Treatment with hCG did not result in more ($P = 0.20$) cows ovulating than treatment with GnRH. Treatment with hCG or GnRH resulted in more ($P < 0.001$) cows ovulating than treatment with saline. Percentage of cows having at least 1 new CL by 7 days after treatment is summarized in Figure 4. Among nonpregnant cows, no difference was detected between hCG and GnRH treatments in the appearance of new CL. Treatment with hCG ($P = 0.07$) tended to produce more accessory CL in pregnant cows, as indicated by a treatment × pregnancy status interaction (Figure 4).

Blood samples were collected from 486 cows at the time of treatment 7 days before pregnancy diagnosis and again at pregnancy diagnosis (d 0). Table 3 summarizes blood serum concentrations of progesterone 7 days after treatment based on treatment, pregnancy status, and number of CL. Concentrations of progesterone 7 days after treatment were adjusted for concentrations of progesterone before treatment. Treatment had no effect on concentrations of progesterone. As expected, pregnant cows (n = 166) had greater ($P < 0.001$) concentrations of progesterone than nonpregnant cows (n = 320). Concentrations of progesterone increased as the number of CL present at collection of the second blood sample increased from 0 to ≥ 2 CL. Stage post-AI (n = 2) did not affect concentrations of progesterone for cows in which treatments were initiated at days 22 to 28 or days 29 to 35.

In summary, no difference was detected in pregnancy survival among cows treated 7 days before pregnancy diagnosis with hCG and those left as untreated controls. Cows treated with GnRH, however, tended to have reduced pregnancy survival compared with controls. Herd had an effect on survival, and a treatment × herd interaction occurred (Exp. 2). Pregnancy rate for GnRH-treated cows tended to differ from that of controls. For every 10-kg increase in test-day milk weight, a 2.2% decrease in pregnancy rate was detected. A treatment × herd interaction occurred as herds 1 and 4 and herds 2 and 3 responded similarly to treatments (Exp. 1). Treatment 7 days before pregnancy diagnosis with hCG or GnRH resulted in a similar number of induced ovulations. Both treatments induced more accessory CL than treatment with saline. Among pregnant cows treated, however, hCG tended to produce more ovulations than GnRH or saline. Treatment had no effect on concentrations of progesterone (Exp. 2).

Table 1. Pregnancy Survival 4 to 9 Weeks After Initial Pregnancy Diagnosis by Treatment in Response to Postpartum Insemination Number (Exp. 1)

Treatment[1]	Postpartum insemination number			
	1	2	≥ 3	Total
	-------------------------------------- % (no.) --------------------------------------			
hCG	91.1 (158)	93.5 (78)	95.7 (184)	93.6 (420)
GnRH	90.8 (152)	94.2 (69)	94.3 (192)	93.0[a] (413)
Control	94.6 (147)	98.4 (63)	94.8 (193)	95.3 (403)
Total	92.1 (457)	95.2 (210)	94.9 (569)	

[a]Tended ($P = 0.06$) to differ from control.
[1]Cows were treated once 7 days before pregnancy diagnosis (days 23 to 38) with hCG, GnRH, or served as untreated controls.

Table 2. Resynchronized Pregnancy Rate by Treatment in Response to Postpartum Insemination Number (Exp. 1)

Treatment[1]	Postpartum insemination number				
	2	3	4	≥ 5	Total
	-------------------------------------- % (no.) --------------------------------------				
hCG	14.9 (161)	19.5 (118)	8.9 (79)	19.1 (183)	16.5 (541)
GnRH	19.2 (198)	19.6 (143)	15.7 (115)	17.0 (247)	17.9[a] (703)
Control	11.8 (144)	13.7 (102)	12.4 (89)	13.5 (170)	12.9 (505)
Total	15.7 (503)	17.9 (363)	12.7 (283)	16.7 (600)	

[a]Tended ($P = 0.08$) to differ from saline.
[1]Cows were treated once with hCG, GnRH, or served as untreated controls 7days before pregnancy diagnosis.

Table 3. Blood Serum Concentrations of Progesterone 7 Days After Treatment Based on Pregnancy Status and Number of Corpora Lutea (CL) at Time of Treatment (Exp. 3)

Item	No. of cows	LS means ± SE	Raw means
		------------------- ng/mL -------------------	
Treatment			
hCG	154	4.7 ± 0.3	5.1
GnRH	151	4.6 ± 0.3	5.4
Control	181	5.1 ± 0.3	5.1
Pregnancy status			
No	320	3.8 [a] ± 0.2	3.7
Yes	166	5.8 [b] ± 0.3	8.1
Corpora lutea, no.			
0	69	2.0 [a] ± 0.4	0.6
1	232	5.6 [b] ± 0.2	5.2
≥ 2	185	6.7 [c] ± 0.2	6.9

[a,b,c] Means having different superscript letters within item differ ($P < 0.001$).

[1] Adjusted for concentrations of progesterone at the time of treatment.

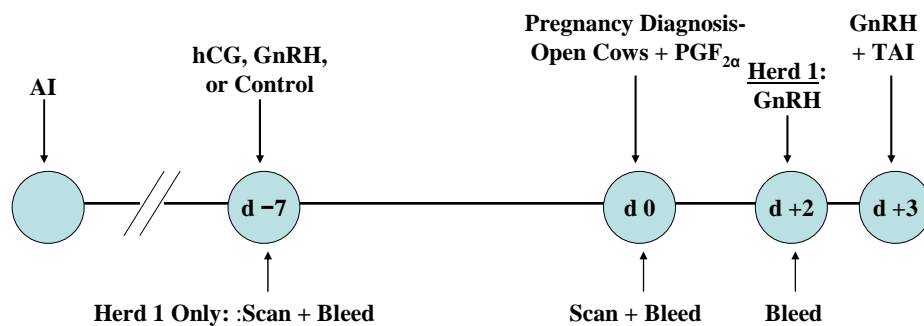[2] Cows were treated once with hCG, GnRH, or served as untreated controls 7 days before pregnancy diagnosis.

Figure 1. Experimental Design. AI = artificial insemination; TAI = timed AI; GnRH = gondadotropin releasing hormone; $PGF_{2\alpha}$ = prostaglandin $F_{2\alpha}$; and hCG = human chorionic gonadotropin.
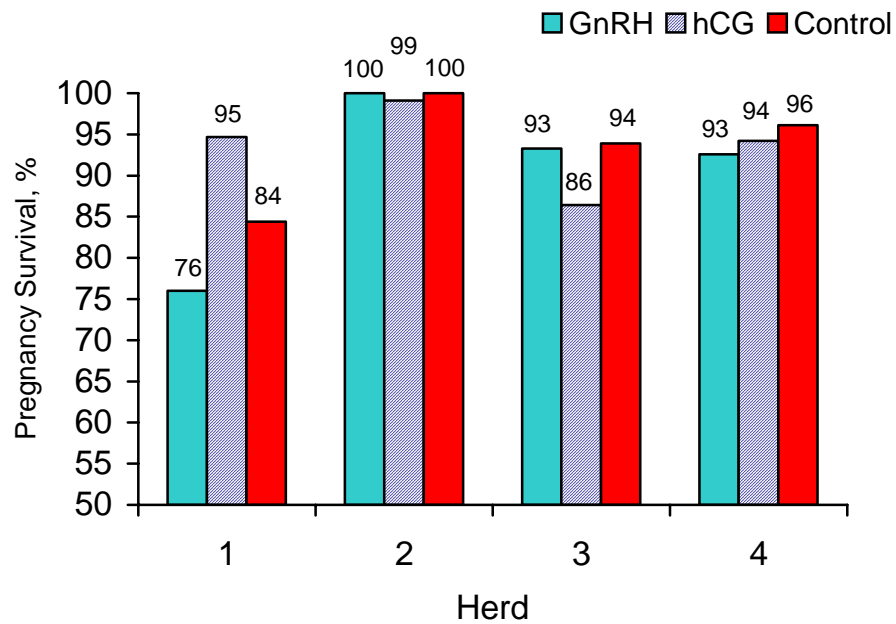
Figure 2. Pregnancy Survival in Lactating Dairy Cows Treated with GnRH, Untreated Control, or hCG. Cows were treated 7 days before initial pregnancy diagnosis (Exp. 1). Pregnancy survival was determined 4 to 9 weeks after initial pregnancy diagnosis. A treatment × herd interaction ($P = 0.004$) was detected.
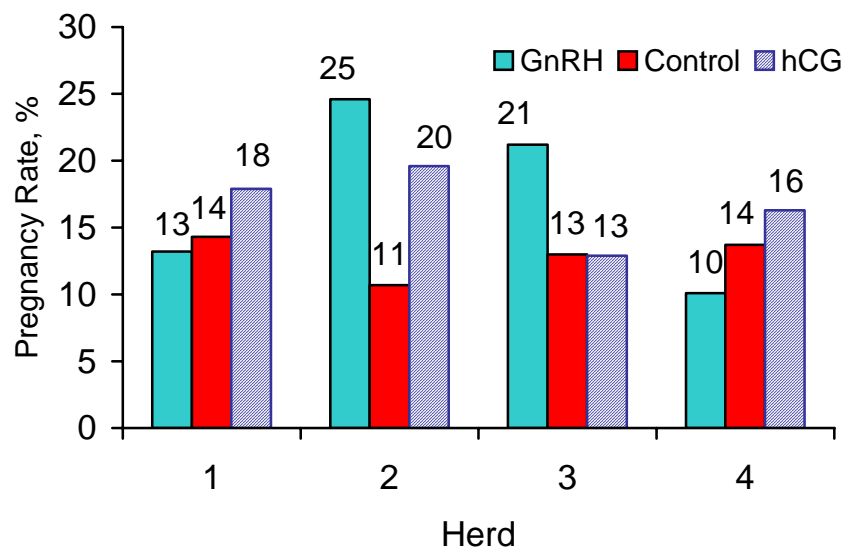

Figure 3 . Pregnancy Rate in Lactating Dairy Cattle Treated with GnRH, Untreated Control, or hCG 7 Days Before Not-pregnant Diagnosis (Exp. 1). A treatment × herd interaction ($P < 0.05$) was detected.
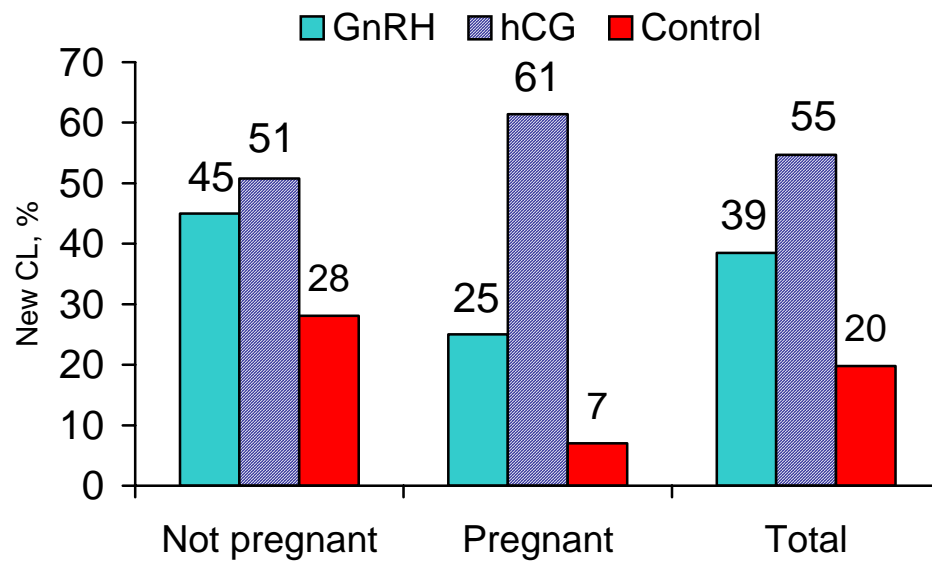
Figure 4. Percentage of Cows Having at Least 1 New Corpus Luteum (CL) by 7 Days After Treatment with GnRH, hCG, or Saline (Exp. 2). A tendency ($P = 0.07$) for a treatment × pregnancy status interaction was detected.