

Resynchronizing Returns to Estrus after a Prior Insemination

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Summary

In two experiments, GnRH and a progesterone insert (CIDR) were applied to cows after insemination (day 0) to reprogram or synchronize the returning estrus of cows that failed to conceive to that previous insemination. The combination of GnRH (day 14) and a CIDR insert (days 17 to 24) in experiment 1 (n = 347 cows) increased the proportion of nonpregnant cows returning to estrus before pregnancy diagnosis on day 32 by 7.4 percentage points, increased the synchrony of their return by 24.4 percentage points, but delayed that return by 2.3 days compared with controls. In experiment 2 (n = 863 cows), use of GnRH alone (day 7), a CIDR insert alone (days 14 to 21), or in combination, failed to increase the proportion of nonpregnant cows in estrus before pregnancy diagnosis on day 32, but cows receiving the CIDR insert had increased synchrony of estrus by 24 to 34 percentage points compared with cows that did not receive a CIDR insert. In both experiments, the treatment of cows with GnRH or GnRH + CIDR insert increased the pretreatment pregnancy rate by 7.1 to 9.5 percentage points. We conclude that administering GnRH with or without a CIDR insert to resynchronize returns to estrus did not significantly improve the proportion of nonpregnant cows reinseminated before pregnancy diagnosis.

Introduction

Although applying various fixed-time insemination programs are critical components of reproductive management of non-pregnant cows, fewer cows would require such intervention if more cows were identified in estrus before pregnancy diagnosis. Detection of returns to estrus from 18 to 24 days after a prior insemination is a labor-intensive method to determine previous pregnancy failure in dairy cattle. Detection of spontaneous returns to estrus, however, is a substantial challenge for dairy herds, with approximately 40 to 60% of non-pregnant cows not reinseminated before pregnancy diagnosis. Efforts to increase the percentage of accurately diagnosed returns to estrus could reduce inter-insemination intervals and promote more efficient use of labor.

Attempts to resynchronize the first eligible estrus in previously inseminated cows have met with limited success because variation in the duration of the estrous cycle after a previous insemination is quite large. Dairy cows with two follicular waves per cycle have shorter estrous cycles, with the ovulatory follicle being both larger and older compared with cows having three follicular waves before insemination. Furthermore, in cows with three follicular waves, luteal function was extended, the peak in plasma progesterone

occurred later in the estrous cycle, and conception rate was greater compared with two-wave cows. Estrus-cycle duration could be reprogrammed via GnRH administration to ovulate the second-wave dominant follicle and cause cows to develop a third follicular wave before returning to estrus (experiment 1). Administering GnRH earlier on day 7, for example, could ovulate a first-wave dominant follicle and form an accessory corpus luteum (experiment 2).

Applying a progesterone insert on day 13 or 14 for 7 days, neither harmed nor benefited ongoing pregnancies, but synchronized returns to estrus in nonpregnant cows. Reprogramming follicular waves to three per cycle in the presence of exogenous progesterone administered at the appropriate time (experiment 1) or inducing an accessory corpus luteum (experiment 2) could synchronize the return to estrus for cows previously inseminated and potentially improve conception rate for cows that are reinseminated.

The hypothesis tested in two experiments was that the application of designed treatments to synchronize estrus in nonpregnant previously inseminated cows leads to reduced inter-insemination intervals and enhanced estrus-detection of nonpregnant cows before pregnancy diagnosis on day 32 after insemination.

Our objective was to reprogram follicular waves in previously inseminated cows by exposing them to GnRH and then controlling return to estrus with exogenous progesterone. We determined the inter-insemination intervals of cows returning to estrus before and after pregnancy diagnosis on day 32 that followed a prior insemination and a subsequent resynchronization treatment. As part of those treatments pre- and post-treatment pregnancy rates also were monitored.

Experimental Procedures

We enrolled lactating Holstein cows in two experiments at the Kansas State University Dairy Teaching and Research Center, Manhattan, KS. Cows were housed in free stalls with overhead roofs and fed a total mixed diet twice or thrice (summer) daily, calculated to meet nutrient requirements for lactating dairy cows producing 110 lb of 3.5% milk fat. The diet consisted of alfalfa hay, corn silage, triticale-sweet clover silage, soybean meal, whole cottonseed, ground corn grain, corn-gluten feed, vitamins, and minerals. Cows were milked thrice daily.

Experiment 1

To reprogram follicular waves from two to three in conjunction with the application of a progesterone insert after a previous AI (day 0), 347 lactating cows were assigned randomly to two treatments during 13 months (June 2017 through June 2018) in 48 weekly clusters. Treatments were: (1) control; or (2) GnRH + CIDR: 200- μ g GnRH on day 14 plus a progesterone (1.38 g) insert applied from d 17 through 24. Criterion for enrollment on day 14 required each cow to have a corpus luteum greater than 20 mm in diameter with bright echogenicity consistent with a functional corpus luteum.

Cows were fit previously with CowManager SensOor ear tags (Agis, Harmelen, the Netherlands) to monitor estrus. Determinations of estrus were diagnosed by the soft-

ware “heat alerts.” Insemination was based on the activity monitor alerts in addition to visual observation of standing-to-be mounted activity. Pregnancy diagnosis occurred weekly (day 32; range in days since insemination was days 30 to 36). Cows diagnosed not pregnant at day 32 ($n = 230$) bearing a CL > 20 mm were treated immediately i.m. with a Short Synch procedure consisting of prostaglandin F_{2a} (2 mL Lutalyse HighCon) and a second dose administered 24 hours later, followed by GnRH (2 mL Factrel) at 56 h after the not-pregnant diagnosis, and insemination 12 to 16 hours later.

Experiment 2

A second experiment was conducted during 24 months (July 2018 through June 2020) to examine at each component of a GnRH + CIDR combination treatment. Lactating dairy cows ($n = 863$) were assigned randomly to four treatments on day 7 after insemination. Treatments were: (1) control; (2) GnRH: 100 μ g GnRH (2 mL Factrel) on day 7; (3) CIDR: insertion of a CIDR on day 14 for 7 days and removed on day 21; or (4) GnRH + CIDR (G + C): combination of the latter two treatments. As for experiment 1, eligibility for enrollment on day 7 required each cow to have a CL greater than 20 mm in diameter with bright echogenicity consistent with a functional CL.

Cows were fit previously with CowManager SensOor ear tags to monitor estrus. Inseminations and pregnancy diagnosis occurred as described for experiment 1. Cows diagnosed not pregnant at day 32 ($n = 544$) bearing a CL > 20 mm were treated immediately i.m. with a Short Synch procedure as in experiment 1.

Results and Discussion

Experiment 1

Simple univariate characteristics of cows enrolled in experiment 1 are as follows. The average number of inseminations at enrollment was 2.0 ± 1.7 (mean \pm SD; range of 1 to 7) with an average 106 ± 49 days in milk (DIM; range of 63 to 280). Body condition scores (1 = thin and 5 = obese) averaged 2.6 ± 0.4 (range of 1.50 to 4.00). Parity of enrolled cows ranged from 1 to 5 with an average of 2.0 ± 1.1 (mean \pm SD) and the pre-enrollment DHI-test day energy-corrected milk averaged 49.0 ± 9.7 kg/d (108 \pm 21.4 lb) with a range of 25.4 to 70.3 kg/day (61.1 to 184.8 lb).

Although numerically greater by 7.3 percentage points, the pretreatment pregnancy rate of cows receiving either the GnRH + CIDR treatment did not differ ($P = 0.15$; Table 1) from controls, but was greater ($P = 0.02$) during the colder than hotter months (39.0 vs. 26.8%). Embryo loss did not differ between controls (3/51; 5.9%) and cows exposed to the GnRH + CIDR treatment (7/66; 10.6%).

The proportion of cows detected in estrus before pregnancy diagnosis was only numerically greater ($P = 0.13$) for GnRH + CIDR compared with control cows (59.5 vs. 52.1%), respectively. Greater synchrony of estrus occurred as more ($P < 0.01$) GnRH + CIDR cows (72.7%) were in estrus on days 26 to 27 compared with only 48.3% of controls on days 21 to 24 (Figure 1A). The pattern of distribution of cows detected in estrus differed ($P < 0.05$) between treatments with different peak and median days to estrus as shown in the survival curve (Figure 1B). Proportion of returning cows was greater ($P = 0.02$) for primiparous than for multiparous cows (65.4 vs. 48.7%),

respectively, but an interaction of treatment and parity also was detected. Although treatment did not affect the proportion of multiparous cows returning to estrus compared with controls (46.2 vs. 51.2%), respectively; among primiparous cows, GnRH + CIDR increased the percentage of returns to estrus compared with the control (76.2 vs. 52.8%). The risk of a longer inter-insemination interval was greater ($P < 0.001$) for GnRH + CIDR-treated cows than for controls (hazard ratio = 1.985; 1.39 to 2.84).

In a subset of 100 cows detected in estrus before pregnancy diagnosis, and based on the CowSensOor accelerometer data, duration of estrus was greater ($P < 0.05$) in GnRH + CIDR cows than in control cows (14.3 ± 0.7 vs. 12.5 ± 0.7 h). In contrast, intensity of estrus was only numerically ($P = 0.13$) greater for GnRH + CIDR than control cows (5.5 ± 0.2 vs. 5.1 ± 0.2 units). For cows detected in estrus before pregnancy diagnosis, the inter-insemination interval was greater ($P < 0.01$) in GnRH + CIDR than control cows, but the subsequent pregnancy rate did not differ between treatments (Table 1).

For cows not detected in estrus before pregnancy diagnosis and diagnosed not pregnant, neither the inter-insemination interval nor the subsequent pregnancy rate after a fixed-time insemination differed between treatments (Table 1). Overall, for all cows regardless of when reinsemination occurred relative to pregnancy diagnosis, neither inter-insemination interval nor pregnancy rate differed between treatments (Table 1). The overall inter-insemination interval was greater ($P = 0.03$) for multiparous than primiparous cows (31.5 ± 0.7 vs. 29.4 ± 0.7 d), respectively. No interactions of parity and treatment were detected for any of the outcomes in Table 1.

Experiment 2

Average number of inseminations at enrollment was 1.7 ± 3.7 (mean \pm SD; range of 1 to 8) and cows averaged 108 ± 50 DIM (range of 57 to 367). Body condition scores averaged 2.4 ± 0.4 (range of 2.0 to 4.0). Parity of enrolled cows ranged from 1 to 6 with an average of 2.0 ± 1.1 (mean \pm SD) and the most recent DHI-test day energy-corrected milk averaged 103.5 ± 17.9 lb/day with a range of 56 to 155 lb/day.

Cows treated with GnRH on d 7 (GnRH and GnRH + CIDR treatments) tended ($P = 0.08$) to have greater pretreatment pregnancy rate (41.3%; 182/441) compared with cows not treated with GnRH (35.2%; 155/440). Neither the main effect of CIDR nor the interaction between GnRH and CIDR affected pretreatment pregnancy rate (Table 2). In addition, season ($P = 0.32$) and parity ($P = 0.57$) did not affect pregnancy rate. Embryo loss resulting from the pretreatment inseminations did not differ between controls (8/74; 10.8%), CIDR (2/81; 2.5%), GnRH (7/89; 7.9%), and cows exposed to the GnRH + CIDR treatment (7/93; 7.5%).

The proportion of cows detected in estrus before pregnancy diagnosis on day 32 did not differ among treatments even though the percentages ranged from 50 to 60% (Table 2). Greater synchrony of estrus was detected in those cows receiving a CIDR insert as more cows ($P < 0.05$) were in estrus on d 21 through 24 compared with cows not receiving a CIDR insert (Figure 2A). Pattern of distribution of nonpregnant cows returning to estrus after the pretreatment insemination did not differ between cows receiving a CIDR insert (CIDR and GnRH + CIDR treatments) and did not differ between

controls and GnRH-treated cows, but distributions of non-CIDR-treated cows differed from CIDR-treated and was delayed as shown in the survival curve (Figure 2B). Proportion of cows detected in estrus before pregnancy diagnosis was greater ($P = 0.04$) for primiparous than for multiparous cows (61.2 vs. 52.7%), respectively. Furthermore, more cows tended ($P = 0.06$) to be detected in estrus during the colder months (58.1%; 194/334) compared with the hotter months (53.2%; 107/201).

The risk of a longer inter-insemination interval was not different ($P = 0.34$) for cows exposed to the CIDR compared with those not treated with a CIDR (HR = 0.89; 0.71 to 1.12). Median inter-insemination interval was 23 days (mean = 23.3 ± 0.2 days) for cows receiving a CIDR insert and 22 days (mean = 22.5 ± 0.3 days) for cows not receiving the CIDR insert.

For cows detected in estrus before pregnancy diagnosis, the inter-insemination interval was approximately 1 d shorter ($P = 0.04$) for cows not receiving a CIDR insert compared with those treated with a CIDR insert, but subsequent pregnancy rate did not differ between treatments (Table 2). In addition, the inter-insemination interval tended ($P = 0.07$) to be shorter in primiparous compared with multiparous cows (22.6 ± 0.5 vs. 23.4 ± 0.3 days), respectively. Moreover, pregnancy rate of cows detected in estrus was greater ($P = 0.04$) in primiparous cows (29.5%; 25/84) than in multiparous cows (18.9%; 27/144).

For cows not detected in estrus before pregnancy diagnosis and diagnosed not pregnant, the inter-insemination interval did not differ among treatments (Table 2), but the subsequent pregnancy rate after a fixed-time insemination was greater ($P = 0.04$) for cows in treatments that received GnRH (27.2%; 31/114) compared with cows not receiving GnRH (17.5%; 20/114). In addition, the inter-insemination interval for non-pregnant cows reinseminated after pregnancy diagnosis was shorter ($P = 0.02$) in primiparous compared with multiparous cows (36.2 ± 0.5 vs. 37.5 ± 0.4 days), respectively.

Overall, for all cows regardless of when reinsemination occurred relative to pregnancy diagnosis, neither inter-insemination interval nor pregnancy rate differed among treatments (Table 2). The pregnancy rate, however, tended ($P = 0.09$) to be greater in cows receiving GnRH (26.8%; 67/250) compared with cows not receiving GnRH (20.8%; 56/269). Overall, inter-insemination interval was shorter ($P < 0.01$) in primiparous compared with multiparous cows (27.9 ± 0.5 vs. 30.3 ± 0.5 days), respectively. In addition, the overall pregnancy rate for all reinseminated cows was greater ($P < 0.01$) in primiparous (28.4%; 61/215) compared with multiparous cows (20.4%; 62/304), respectively. No interactions of parity and treatment were detected for any of the outcomes in Table 2.

Discussion

In both experiments, previously inseminated cows treated with either GnRH alone or both GnRH + CIDR compared with the control had numerically improved pregnancy rates compared to the control by 7.3 percentage points (GnRH + CIDR in experiment 1), 7.1 percentage points (GnRH in experiment 2), and 9.5 (G + C in experiment 2) percentage points. This finding is consistent with other studies and shows a benefit of

providing a second corpus luteum and more endogenous or supplemental progesterone during early pregnancy. The cost associated with applying both GnRH and a CIDR insert could vary from \$12 to \$14 per cow in addition to the labor cost. At a cost of \$12 per cow and an increase of 8 pregnancies per 100 cows treated, the value of each pregnancy must be worth more than \$66.67 per cow to cover production costs alone. Average value of a new pregnancy was \$278 for a Holstein cow in the United States in 2006. The value of a new pregnancy increased with days in milk early in lactation but typically decreased later in lactation. Relatively high-producing cows and first-lactation cows reached greater values, and their values peaked later in lactation. The average cost of a pregnancy loss (abortion) was \$555. The value of pregnancy and optimal breeding decisions for individual cows greatly depend on the predicted daily milk yield for the remaining period of lactation.

We numerically increased the percentage of nonpregnant cows detected in estrus (7.4 percentage points) before pregnancy diagnosis by the GnRH + CIDR treatment in experiment 1, but were unsuccessful in experiment 2 with any of the treatments administered at a different time. In contrast, in both experiments, returns to estrus were more synchronous with more cows in estrus at the same time. The inter-insemination intervals of cows in experiments 1 and 2 were increased by 2.3 days or 1 day, respectively, because of the progesterone supplementation.

In experiment 2, treatment with GnRH on day 7 resulted in improved pregnancy rates in cows that were detected not pregnant at day 32 and received the timed insemination 3 days after the not-pregnant diagnosis.

Conclusions

We did not prove our hypothesis that the designed treatments would increase the proportion of nonpregnant cows in estrus before the first pregnancy diagnosis after a prior insemination. Although nearly 60% of nonpregnant cows treated with GnRH + CIDR in experiment 1 were detected in estrus, the synchrony of their expressed estrus also was improved. Treatments in experiment 2 failed to increase the returns to estrus, but did increase the synchrony of those returns. Given the costs associated with such treatments, attempts to resynchronize returns to estrus in previously inseminated cows are not advisable.

Table 1. Pretreatment pregnancy rate, subsequent inter-insemination intervals, characteristics of estrus (LSM \pm SEM), and pregnancy rate for cows returning to estrus before or after pregnancy diagnosis (experiment 1)

Item	Treatment ¹		P-value
	Control	GnRH + CIDR	
Pretreatment pregnancy rate, %	30.0 (51/170)	37.3 (66/177)	0.15
Detected in estrus, ² %	52.1 (62/119)	59.5 (66/111)	0.13
Inter-insemination interval, d	24.0 \pm 0.3	26.3 \pm 0.3	<0.01
Return pregnancy rate, %	29.0 (18/62)	27.3 (18/66)	0.85
Not detected in estrus ³			
Inter-insemination interval, d	37.0 \pm 0.7 (57)	37.7 \pm 0.9 (45)	0.57
Return pregnancy rate, %	22.8 (13/57)	26.7 (12/45)	0.64
Overall			
Inter-insemination interval, d	30.2 \pm 0.7	30.7 \pm 0.7	0.62
Pregnancy rate, %	26.1 (31/119)	27.0 (30/111)	0.80

¹Cows were controls or treated with 200 μ g GnRH on day 14 and received a CIDR insert between days 17 and 24 after insemination (d 0).

²Detected in estrus visually or by SensOor tags and reinseminated before pregnancy diagnosis on day 32.

³Not detected in estrus before pregnancy diagnosis on day 32 and reinseminated by fixed-time AI after a non-pregnancy diagnosis.

Table 2. Pretreatment pregnancy rate and inter-insemination interval (LSM \pm SEM) and pregnancy risk after resynchronization with GnRH, progesterone, or both (experiment 2)

Item	Treatment ¹				P-value		
	Control	CIDR	GnRH	G + C	GnRH	CIDR	G \times C
Pretreatment pregnancy rate, %	33.0 (74/224)	37.5 (81/216)	40.1 (89/222)	42.5 (93/219)	0.08	0.44	0.81
Detected in estrus, ² %	59.5 (89/148)	55.6 (75/135)	50.0 (64/128)	59.7 (74/124)	0.59	0.59	0.15
Inter-insemination interval, days	22.3 \pm 0.4 (88)	23.3 \pm 0.4 (75)	22.8 \pm 0.5 (64)	23.5 \pm 0.4 (74)	0.46	0.04	0.70
Return pregnancy rate, %	25.3 (21/83)	20.8 (15/72)	29.0 (18/62)	24.3 (18/74)	0.52	0.36	0.89
Not detected in estrus ³							
Inter-insemination interval, days	36.9 \pm 0.5 (61)	37.4 \pm 0.6 (60)	36.9 \pm 0.6 (64)	36.2 \pm 0.6 (50)	0.28	0.86	0.30
Return pregnancy rate, %	15.8 (9/57)	19.0 (11/58)	28.1 (18/64)	26.0 (13/50)	0.04	0.99	0.77
Overall							
Inter-insemination interval, days	28.2 \pm 0.7 (149)	29.7 \pm 0.7 (135)	29.8 \pm 0.7 (128)	28.6 \pm 0.7 (124)	0.76	0.82	0.06
Pregnancy rate, %	21.4 (30/140)	20.0 (26/130)	28.6 (36/126)	25.0 (31/124)	0.09	0.39	0.95

¹Control cows were untreated, or cows were treated with 100 μ g GnRH on day 7 post-AI, received progesterone supplementation (CIDR) from days 7 to 14 after insemination (day 0), or both GnRH and CIDR (G + C) treatments. Cows culled before reinsemination (2 controls, 0 CIDR, 5 GnRH, and 2 G + C cows) or before pregnancy diagnosis after reinsemination: (8 controls, 5 CIDR, 2 GnRH, and 0 G + C cows), respectively.

²Detected in estrus visually or by SensOor tags and reinseminated before pregnancy diagnosis on day 32.

³Not detected in estrus before pregnancy diagnosis on day 32 and reinseminated by fixed-time AI after a non-pregnancy diagnosis.

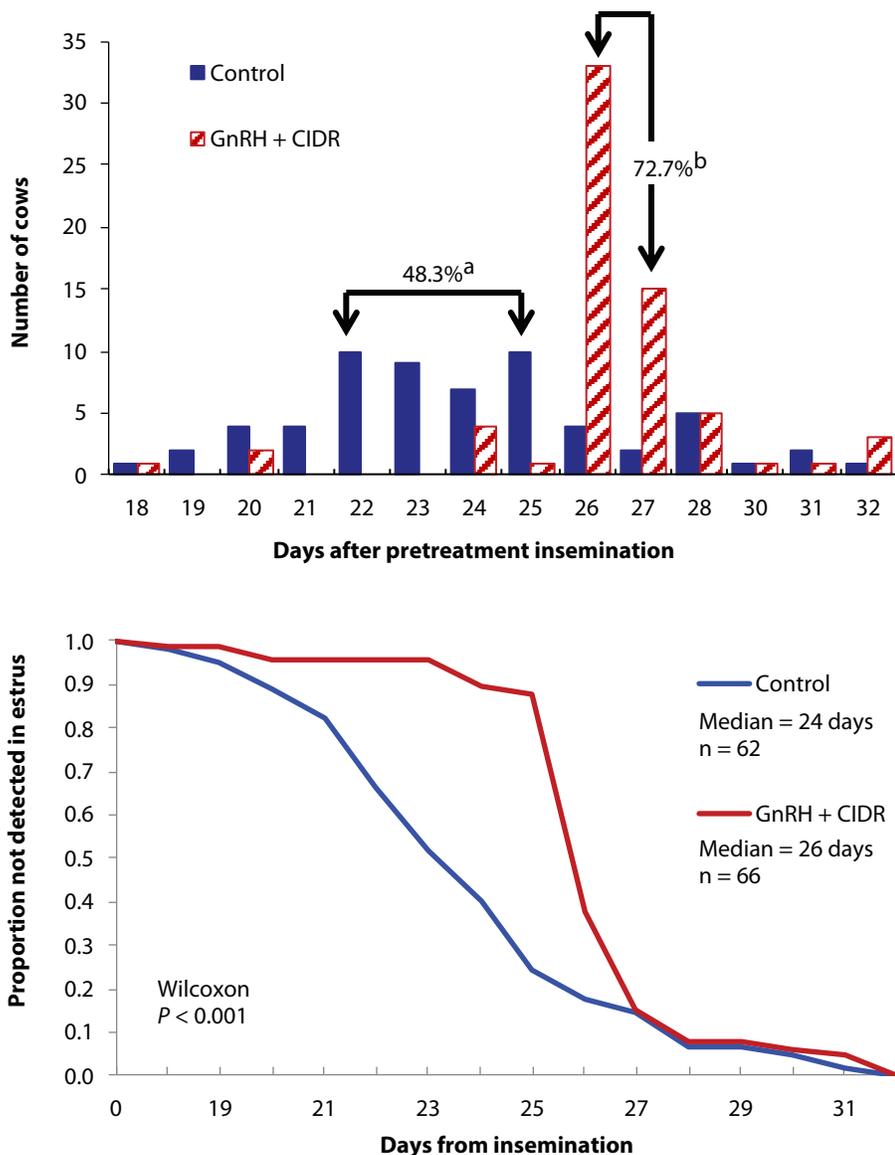


Figure 1. (A) Distribution of nonpregnant cows detected in estrus after a prior insemination and before pregnancy diagnosis on day 32 after insemination (d 0). Control cows were untreated or cows were treated with 200 μ g GnRH on day 14 post-AI and received progesterone supplementation (CIDR insert) from days 17 through 24 after insemination. ^{a,b}Percentage in estrus on days 21 through 24 in the control differed ($P < 0.01$) from that in GnRH + CIDR cows in estrus on days 26 and 27. (B) Survival curve illustrating the proportion of cows not detected in estrus and reinseminated before pregnancy diagnosis on day 32 (experiment 1).

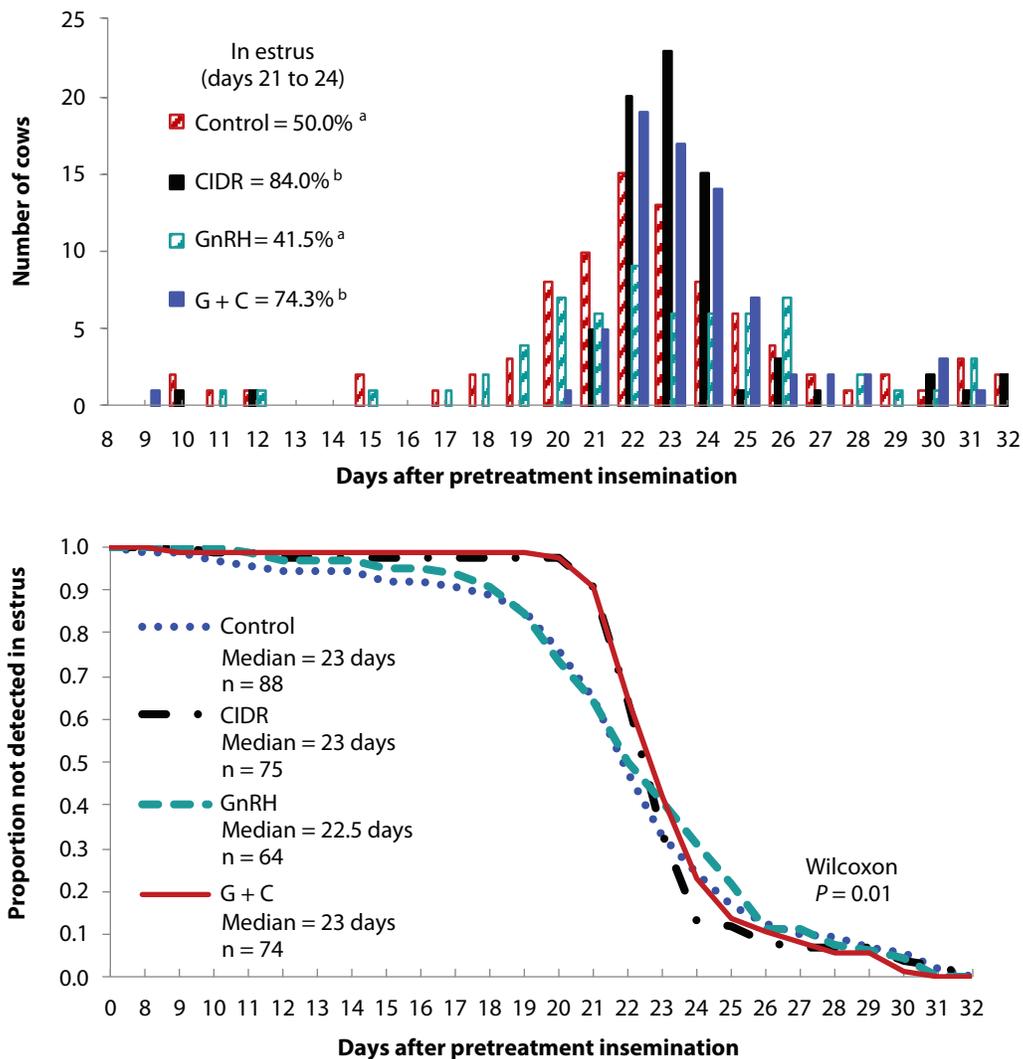


Figure 2. (A) Distribution of nonpregnant cows detected in estrus after the pretreatment insemination (d 0) and before pregnancy diagnosis on day 32 for controls, cows treated with 100 µg GnRH on day 7, cows receiving progesterone supplementation (CIDR) from days 7 through 14, or both GnRH and CIDR (G + C) treatments. ^{a,b}Percentage in estrus on days 21 through 24 differed ($P < 0.01$) among treatments. (B) Survival curve illustrating the proportion of cows detected in estrus and reinseminated before pregnancy diagnosis on day 32 (experiment 2).