Post-breeding Treatments to Improve Fertility in Lactating Dairy Cows[a]

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Abstract

Lactating Holstein cows were assigned randomly to treatments to improve fertility after first postpartum timed artificial insemination (TAI). In Experiment 1, cows received no treatment (C; n = 223); a CIDR insert from five to 12 days after TAI (CIDR; n = 218); or 100 μg of genadotropin releasing hormone (GnRH) five days after TAI (G5; n = 227). In Experiment 2, cows received C (n = 160), G5 (n = 159), or treatment with 100 μg of GnRH seven days after TAI (G7; n = 163). Although treatment did not affect fertility, when data were combined to compare C (n = 383) and G5 (n = 386) treatments, pregnancies per AI (P/AI) tended to be greater for G5 (49.1 %) than C (45.8 %) cows, and this effect resulted from a GnRH treatment by cyclicity status interaction in which P/AI for anovular cows receiving G5 was greater than that for anovular C cows (45.5 vs. 31.1 %). In conclusion, treatment with CIDR inserts after TAI had no effect on fertility, whereas treatment with GnRH five days after TAI improved fertility for anovular, but not for cycling cows.

Résumé

Des vaches Holstein en lactation ont été allouées aléatoirement à des groupes de traitements visant à améliorer la fertilité après la première insémination artificielle (IA) postpartum sur rendez-vous. Dans l'expérience 1, les vaches du groupe C ne recevaient aucun traitement (n = 223), celles du groupe CIDR recevaient un implant vaginal libérant de la progestérone de 5 à 12 jours suivant l'IA sur rendez-vous (n = 218) et celles du groupe G5 recevaient 100 μg de gonadoréline 5 jours suivant l'IA sur rendez-vous (n = 227). Dans l'expérience 2, les vaches du groupe C ne recevaient toujours rien (n = 160) alors que les autres recevaient 100 μg de gonadoréline soit 5 jours (G5, n = 159) ou soit 7 jours (G7, n = 163) suivant l'IA sur rendez-vous. Le traitement n'a pas eu d'effet sur la fertilité, mais lorsque les données ont été combinées pour comparer le groupe témoin (n = 383) et le groupe G5 (n = 386), le taux de conception par IA était marginalement plus élevé chez les vaches du groupe G5 (49.1%) que chez les vaches du groupe C (45.8%). Cette différence provenait d'une interaction entre l'administration de gonadoréline et l'état physiologique de la vache : le taux de conception par IA était plus élevé chez les vaches anovulatoires du groupe G5 que chez les vaches anovulatoires du groupe témoin (45 versus 31.1%). En conclusion, le traitement avec les implants vaginaux libérant de la progestérone n'a pas eu d'effet sur la fertilité alors que le traitement avec administration de gonadoréline 5 jours suivant l'IA augmentait le taux de conception par IA chez les vaches anovulatoires mais pas chez les vaches cycliques.

Introduction

In the decade following the first published report describing Ovsynch, much attention has been devoted to optimizing synchronization and the timing of timed artificial insemination (TAI), whereas less is know about improving fertility after TAI. Fertilization rates during winter in lactating dairy cows were 87%, yet only 52.8% of the subsequent embryos were grades one to three five days after ovulation, suggesting that early embryonic development and embryonic mortality, rather than fertilization failure, are the primary obstacles to achieving reproductive success.[16] Thus, strategies that mitigate early embryonic loss may significantly improve reproductive efficiency.

Gümen *et al*[5] reported that cows retrospectively identified pregnant had greater circulating concentrations of progesterone (P_4) as early as five days after AI to detected estrus, and 11 days after Ovsynch and TAI. Moreover, Mann and Lamming[10] demonstrated that embryonic development is affected by the pattern of secretion and concentration of maternal P_4 after ovulation. Cows with a delayed increase and decreased circulating P_4 after ovulation produced embryos with a compromised capacity to produce the antiluteolytic hormone INF-t. Previous studies have investigated the effect of treatment with genadotropin-releasing hormone (GnRH) after insemination to improve pregnancy rates by inducing ovulation and increasing P_4 through formation of an accessory corpus luteum (CL). In a meta-analysis conducted by Peters *et al*[14] GnRH treatment between 11 and 14 days after AI improved pregnancy rates in only five of 19 studies, but when the data set was limited to six trials with five common variables, GnRH treatment increased conception rate.

Thus, there is evidence for a relationship between maternal P_4 after insemination and fertility. Moreover, data to date do not provide conclusive evidence on the efficacy of post-AI treatments, or cow factors that may affect treatment response. To further investigate the relationship between maternal P_4 and fertility, we examined two treatments that had the potential to increase circulating P_4 after insemination: 1) exogenous administration of GnRH five or seven days after TAI to produce accessory CL, or 2) administration of a CIDR insert containing 1.38 g of P_4 from five to 12 days after TAI. The primary objective of this study was to evaluate fertility and pregnancy loss after first postpartum TAI for cows treated with CIDR inserts or GnRH after TAI. Our hypothesis was that treatments to increase P_4 concentration after TAI would improve PR/AI, possibly through reduced pregnancy loss.

Materials and Methods

Experiments 1 and 2

For Experiments 1 and 2, lactating Holstein dairy cows on a commercial dairy farm located in north-central Wisconsin were enrolled at TAI into the study. Cows were housed in free-stall barns and fed a total mixed ration (TMR) once daily with ad libitum access to feed and water. Cows were milked thrice daily at approximately eight-hour intervals.

Cows were managed as groups to receive hormone injections and TAI on two preselected days of the week (Tuesdays and Thursdays). All cows received a hormonal synchronization protocol (Presynch + Ovsynch) using intramuscular injections of GnRH and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) before first postpartum TAI as follows: $PGF_{2\alpha}$ (day 32 ± 3 and day 46 ± 3), GnRH (day 60 ± 3), $PGF_{2\alpha}$ (day 67 ± 3), and GnRH + TAI (day 69 ± 3) postpartum. Cows were not inseminated after detected estrus during the synchronization program. For Experiment 1, cows were randomized at TAI to each of three treatments: 1) no further treatment (C, n = 223); 2) a CIDR insert from five to 12 days after TAI (CIDR, n = 218); or 3) 100 μg of GnRH five days after TAI (G5, n = 227). For Experiment 2, cows were randomized to each of three treatments to receive C (n = 160) or G5 (n = 159), or 100 μg GnRH seven days after TAI (G7, n = 163).

Assessment Cyclicity Status at Initiation of Ovsynch

Ultrasound examinations, hormone injections and body condition scoring were conducted immediately after milking by the herd veterinarian and herd personnel while cows were restrained in a palpation rail located in the breezeway exiting the milking parlor. At the first GnRH of Ovsynch, cows were classified based on presence or absence of a CL (cycling vs. anovular, respectively) using transrectal ultrasonography as described previously.[3] As part of the routine reproductive management on this dairy, all anovular cows at the first GnRH of Ovsynch received a CIDR insert between the first GnRH and the $PGF_{2\alpha}$ injections of Ovsynch.

For pregnancy diagnosis, visualization of a CL, fluid-filled uterine horn and presence of a conceptus were positive indicators of pregnancy 33 and 61 days after TAI using ultrasound as described previously.[4] The number of cows diagnosed pregnant to TAI, expressed as a percentage of cows within that treatment group receiving TAI, was defined as pregnancies per AI (P/AI). Cows diagnosed pregnant 33 days after TAI were scheduled for a pregnancy recheck using transrectal ultrasound 61 days after TAI, and pregnancy loss was assessed between 33 and 61 days after TAI.

Statistical Analyses

Experiments were conducted using a randomized complete block design.[13] Each week at the initial TAI, cows within a weekly breeding group were blocked according to parity (primiparous vs. multiparous). Within each block, cows were assigned randomly to each of three treatments after first postpartum TAI.

Dichotomous data were analyzed using PROC LOGISTIC of SAS.[17] A multivariate logistical regression model was developed to analyze the effects of the categorical variables treatment, parity (primiparous vs. multiparous), season (spring, summer, winter, or fall) and cyclicity status (cycling vs. anovular) at the first GnRH of Ovsynch, along with the continuous variable body condition score (BCS) at the $PGF_{2\alpha}$ of Ovsynch. In addition, all two-way interactions of the explanatory variables with treatment on P/AI and pregnancy loss were included. For the model analyzing combined data (Experiment 1 vs. 2), treatment and the treatment x experiment interaction were added to the model.

All multivariate logistical regression models were constructed using a backward selection procedure with treatment retained as a fixed factor in each of the models. A Wald statistic criterion of $P < 0.15$ was set for including variables in the model. Odds ratios and 95 % confidence intervals were calculated for significant main effects remaining in the final models. Data are presented as percentages and proportions with P values for main effects and interactions derived from the multivariate logistical regression analysis.

Results and Discussion

Experiment 1

Percentages and proportions for P/AI and pregnancy loss for Experiment 1 are presented in Table 1. Overall, P/AI 33 days after TAI did not differ ($P = 0.20$) among treatments and was 49.8 % (111/223) for C, 55.5 % (126/227) for G5 and 46.8 % (102/218) for CIDR cows, respectively. Similarly, P/AI 61 days after TAI did not differ ($P = 0.18$) among treatments and was 45.5 % (100/220) for C, 50.9 % (115/226) for G5 and 42.4 % (92/217) for CIDR cows, respectively. Pregnancy loss from 33 to 61 days after TAI did not differ ($P = 0.14$) among treatments and was 7.4 % (8/108) for C, 8.0 % (10/125) for G5 and 8.9 % (9/101) for CIDR cows, respectively.

Because estrous cycles were presynchronized using two injections of $PGF_{2\alpha}$ administered 14 days apart with the second $PGF_{2\alpha}$ injection administered 14 days before the first GnRH injection of Ovsynch, we expected that cycling cows should have a midcycle CL at the first GnRH of Presynch + Ovsynch, whereas cows lacking a CL at the first GnRH injection of Presynch + Ovsynch most likely had a delayed resumption of postpartum cyclicity (i.e., were anovular). In another experiment, Silva *et al*[18] compared the presence or absence of a CL at the first GnRH injection of Presynch + Ovsynch to a previously published method for identifying anestrus cows using combinations of high (> 1.0 ng P_4/ml) and low (≤ 1.0 ng P_4/ml) serum P_4 collected at the second $PGF_{2\alpha}$ injection and at the first GnRH injection of Presynch + Ovsynch.[11] Based on data from 863 cows, sensitivity, specificity, positive predictive value and negative predictive value of using transrectal ultrasonography to identify cyclicity status were 85.7, 87.7, 64.7 and 95.9 %, respectively.[18] Disagreements between a single ultrasound and two serum P_4 radioimmunoassays (RIAs) occurred because 9.0 % of all cows had serum P4 ≥ 1.0 ng/ml at the second $PGF_{2\alpha}$ injection of Presynch and < 1.0 ng/ml at the first GnRH injection of Ovsynch. Furthermore, 6.3 % of all cows were classified as cycling because of the presence of a CL using ultrasound, but had serum P4 < 1.0 ng/ml at the first GnRH injection of Ovsynch. Thus, the majority of CL- cows at the first GnRH injection of Presynch + Ovsynch were considered to be anovular for the purposes of discussion in the present study.

In agreement with the present study, others[1,8] failed to observe a treatment effect when GnRH was administered 5 days after TAI. In contrast, Santos *et al*[15] reported an increase in pregnancy rate for lactating dairy cows receiving human chorionic gonadotropin (hCG) five days after AI to estrus, particularly for those cows losing body condition between the time of AI and pregnancy diagnosis. Insertion of a pregesterone-releasing intravaginal device (PRID) containing 1.5 g of P_4 from five to 13 days after first postpartum insemination failed to affect fertility in a limited number of cows.[19] Treatment of anestrous cows with a PRID beginning four or five days after AI also failed to improve fertility.[7] Use of a CIDR insert from 14 to 21 days after AI to resynchronize return to estrus for cows failing to conceive at first AI had a slight but significant negative effect on pregnancy rate to the previous insemination (32.7 vs. 36.7 %).[2]

Experiment 2

Results from Experiment 1 showed no benefit of treating cows with CIDR inserts from five to 12 days after TAI, but a non-statistical trend for a benefit of treating cows with GnRH 5 days after TAI (Table 1). We

Table 1. Effects of cyclicity status at the first GnRH injection of Presynch + Ovsynch on pregnancies per AI (P/AI) and pregnancy loss for lactating Holstein cows in Experiment 1.*

	Treatment						P value		
	Control		GnRH d 5		CIDR				
Item	Anovular	Cycling	Anovular	Cycling	Anovular	Cycling	Treatment	Cyclicity	Interaction
P/AI, % (no./no.)									
33 days	37.3 (22/59)	54.3 (89/164)	51.0 (26/51)	56.8 (100/176)	30.0 (12/40)	50.6 (90/178)	0.20	< 0.01	—
61 days	32.2 (19/59)	50.3 (81/161)	44.0 (22/50)	52.8 (93/176)	22.5 (9/40)	46.9 (83/177)	0.18	< 0.01	—
Pregnancy loss, % (no./no.)	13.6 (3/22)	5.8 (5/86)	12.0 (3/25)	7.0 (7/100)	25.0 (3/12)	6.7 (6/89)	0.14	0.01	—

*Adapted from Sterry *et al*, *J Dairy Sci* 89:4237-4245, 2006.

speculated that the lack of a significant effect of the G5 treatment may have involved a Type II error (declaring no difference between the G5 and C treatments when a difference does exist). Thus, Experiment 2 was initiated immediately after Experiment 1 to continue the C and G5 treatments to further increase the statistical power of the comparison between the C and G5 treatments. Because less than 160 n=160, 159, 163 cows were included in each treatment in Experiment 2, the possibility of Type II errors must be considered when interpreting these data.

As described previously, anovular cows received a CIDR insert during the Presynch + Ovsynch protocol. No differences were detected for P/AI 33 days ($P > 0.10$; C = 51.3 %, 82/160; G5 = 49.7 %, 79/159; G7 = 52.1 %, 85/163), or 61 days after TAI (C = 46.3 %, 74/160; G5 = 46.5 %, 73/157; G7 = 46.9 %, 76/162). A tendency (P = 0.09) was detected, however, for a treatment effect on pregnancy loss from 33 to 61 days after TAI (C = 9.8 %, 8/82; G5 = 5.2 %, 4/77; G7 = 9.5 %, 8/84).

Analysis of G5 and C Treatments from Experiments 1 and 2

When results from G5 and C cows from Experiments 1 and 2 were combined and analyzed, there was a tendency for a GnRH treatment x cyclicity status interaction, in which anovular G5 cows had more P/AI than anovular C cows (Table 2). At 33 days after TAI, anovular G5 cows tended (P = 0.08) to have more P/AI (51.1 %, 46/90), than anovular C cows (37.7 %, 40/106). By 61 days after TAI, there was still a tendency ($P <$ 0.06) for the GnRH treatment x cyclicity status interaction with anovular G5 cows having more P/AI than anovular C cows (45.5 %, 40/88 vs. 31.1 %, 33/106, respectively). Pregnancy loss from 33 to 61 days after TAI, however, did not differ between treatments (9.1 %, 4/44 vs. 17.6 %, 7/40 for G5 and C cows, respectively).

It is important to evaluate the type of synchronization protocol imposed before insemination when comparing reported responses to GnRH treatments administered after AI among studies. In the present study and in Bartolome *et al*[1], all cows were enrolled into the experiment after synchronization of ovulation using Presynch + Ovsynch. Although cyclicity status was not assessed in their study, Bartolome *et al* [1] also failed to detect a treatment effect on P/AI (47.7 vs. 44.4 %; P = 0.11) for cows treated with GnRH five days after TAI. Hanlon *et al*[7] treated cows with hCG five days after AI (no palpable CL and were not observed in estrus before AI) with no subsequent effect on fertility. In that study, however, estrus was synchronized using a CIDR insert and estradiol benzoate, and only cows expressing estrus within the first two days of the breeding period were enrolled into the study. Similarly, Santos *et al* [15] limited enrollment to cows that displayed estrus after treatment with GnRH and $PGF_{2\alpha}$, resulting in 72 % of eligible cows receiving insemination after a detected estrus before treatment with hCG. Because cows in the latter two studies were inseminated after detected estrus, non-cycling cows were either excluded from enrollment, or the synchronization protocol imposed before treatment may have resolved the anestrous condition, thereby confounding the comparison of the effectiveness of GnRH administered after breeding among these studies with the present results.

Effect of Body Condition Score on the Proportion of Anovular Cows

Cows with a high body condition score (BCS) during Presynch + Ovsynch had a greater ($P \leq 0.01$) PR/AI than cows with low BCS (defined as ≤ 2.5) (Table 3). At 33 days after TAI, cows with a high BCS had a PR/AI of 55.2 % (334/605), compared to 39.0 % (64/164) for cows with low BCS (AOR = 1.9; 95 % CI = 1.3 – 2.7). At 61 days

Table 2. Effects of cyclicity status at the first GnRH injection of Presynch + Ovsynch and pregnancies per artificial insemination (P/AI) and pregnancy loss for lactating Holstein cows in Experiments 1 and 2.*

	Treatment						
	Control		GnRH d 5		*P* value		
Variable	Anovular	Cycling	Anovular	Cycling	Treatment	Cyclicity	Interaction
P/AI, % (no./no.)							
33 days	37.7 (40/106)	55.2 (153/277)	51.1 (46/90)	54.3 (159/293)	0.08	0.02	0.08
61 days	31.1 (33/106)	51.5 (141/274)	45.5 (40/88)	50.7 (148/292)	0.11	< 0.01	< 0.06
Pregnancy loss, % (no./no.)	17.6 (7/40)	6.0 (9/150)	9.1 (4/44)	6.3 (10/158)	0.15	0.02	—

*Adapted from Sterry *et al*, *J Dairy Sci* 89:4237-4245, 2006.

Table 3. Body condition scores (BCS) at the PGF_{2a} injection of Ovsynch and pregnancy rate per AI (PR/AI) and pregnancy loss for lactating Holstein cows for treatments C and G5 from Experiments 1 and 2.*

	BCS at the PGF_{2a} injection of Ovsynch				
Variable	≤ 2.5	> 2.5	*P* Value		
PR/AI, % (no./no.)			Treatment	BCS	Interaction
33 days	39.0 (64/164)	55.2 (334/605)	0.125	< 0.001	0.682
61 days	36.4 (59/162)	50.4 (303/601)	0.089	0.003	0.507
Pregnancy loss, % (no./no.)	4.8 (3/62)	8.2 (27/330)	0.463	0.117	0.468

*Adapted from Sterry *et al*, *J Dairy Sci* 89:4237-4245, 2006.

after TAI cows with greater BCS had a greater PR/AI, 50.4 % (303/601) versus 36.4 % (59/162) for cows with lesser BCS (AOR = 1.7; 95 % CI = 1.2 – 2.5). Finally, there was a tendency ($P = 0.12$) for cows with low BCS to lose fewer pregnancies (4.8 [3/62] vs. 8.2 % [27/330]). Our definition of low BCS differed from < 2.5 used by Moriera *et al*[12] due to the low number of cows with BCS < 2.5 in the present study (53/760). Still, the findings of Moriera *et al*[12] agree with our study in that cows classified as having low BCS had reduced fertility at 27 days (18.1 vs. 33.8 %) and 45 days (11.1 vs. 25.6 %) after TAI.

Although anovulation and low BCS result in reduced fertility, the two classifications identify two distinct subpopulations. A greater proportion of cows classified as having a BCS < 2.5 were also classified as anovular, nearly 40% (24/61). Overall, 28 % (49/178) of cows with low BCS were anovular, compared to 20 % for BCS ≥ 2.75 (99/487) (Figure 1). Because of this, identifying anovular cows by BCS ≤ 2.5 would identify only one third of all anovular cows, due to the large number of cows with BCS greater than 2.5 (487/665). Lopez *et al*[9] reported a linear relationship between BCS and anovulation, yet 63.1 % of anovular cows had a BSC ≥ 2.75. Thus, mechanisms other than low BCS likely play a role in the cause of anovulation for the majority of anovular cows.[9]

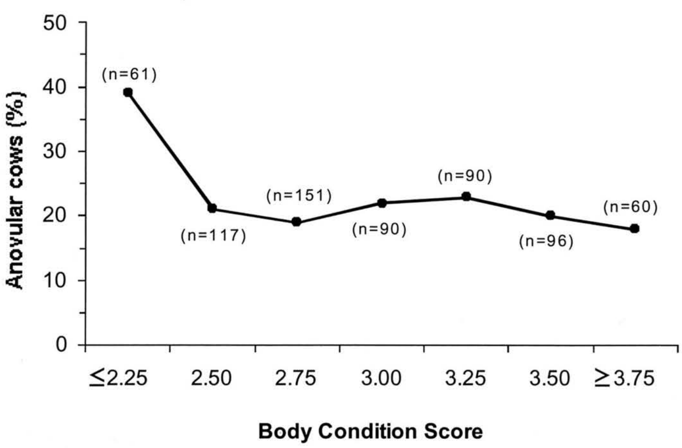

Figure 1. Proportion of cows classified as anovular by body condition score. Cows received their first postpartum timed artificial insemination (TAI) after synchronization of ovulation using Presynch + Ovsynch. To assess cyclicity status, cows were classified as either having or lacking an accessory corpus luteum at the first GnRH injection of Presynch + Ovsynch using transrectal ultrasonography.

Conclusions

Overall, treatment with CIDR inserts from day five to 12 or GnRH 7 days after TAI failed to improve fertility or reduce pregnancy loss in lactating dairy cows. When data were combined from Experiment 1 and 2, treatment with GnRH 5 days after TAI tended to increase P/AI. The increase in P/AI for the G5 treatment occurred because of a GnRH treatment x cyclicity status interaction, in which anovular cows treated with GnRH five days after TAI tended to have more P/AI than untreated anovular cows. Further research is needed to develop practical methods for identifying cows with delayed postpartum cyclicity and to identify the mechanisms by which the G5 treatment may improve fertility in lactating dairy cows.

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