PEER REVIEWED

Effect of New and Reused CIDRs on Serum Progesterone Concentrations in Lactating Dairy Cows

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Abstract

Despite the common practice of re-using a popular intravaginal progesterone releasing device by United States cattle producers, there is little published scientific evidence whether this practice is effective as an aid in synchronizing estrus. We compared baseline serum progesterone concentrations in healthy lactating dairy cows to serum progesterone concentrations following insertion of new intravaginal progesterone releasing devices (1.38 g CIDR) and to concentrations following reuse of the same intravaginal progesterone releasing devices in the same cows. Under the conditions of the study, there was a significant decrease in serum progesterone concentrations following insertion of used CIDRs when compared to serum progesterone concentrations following insertion of new CIDRs. Serum progesterone concentrations associated with used CIDRs were statistically similar to baseline progesterone concentrations obtained from study cows before new CIDR insertion. Results of this study indicate there was either significant loss of progesterone in the CIDR insert after one use or there was decreased absorption of progesterone available from the used CIDRs. Based on the findings of the current study, reuse of 1.38 g CIDRs to aid in synchronization of estrus in lactating dairy cattle cannot be recommended.

Keywords: bovine, CIDR, progesterone, estrus synchronization

Résumé

Bien que la réutilisation d'un dispositif intravaginal populaire libérant de la progestérone soit fréquente chez les producteurs bovins des États-Unis, il existe peu de preuve scientifique établie supportant l'efficacité de cette pratique pour synchroniser les œstrus. Nous avons comparé le niveau basal de la concentration sérique de progestérone chez des vaches laitières en santé pendant la lactation aux concentrations sériques de progestérone suite à l'insertion d'un nouveau dispositif intravaginal libérant de la progestérone (1.38 g CIDR) et à celles suivant la réutilisation des mêmes dispositifs intravaginaux libérant de la progestérone dans les mêmes vaches. Dans les conditions de cette étude, la concentration sérique de la progestérone suivant l'insertion des CIDR réutilisés était plus basse que celle obtenue suite à l'insertion d'un nouveau dispositif. La concentration sérique de progestérone obtenue suite à l'insertion des CIDR réutilisés n'était pas statistiquement différente de la concentration basale obtenue chez les vaches avant l'insertion des dispositifs. Les résultats de cette étude indiquent que la réutilisation des CIDR engendre soit une diminution significative de la progestérone dans le dispositif ou soit une absorption moindre de la progestérone disponible après réutilisation. En se basant sur les résultats de cette étude, la réutilisation des dispositifs de 1.38 g pour faciliter la synchronisation des œstrus chez les vaches laitières en lactation n'est pas recommandable.

Introduction

Intravaginal progesterone-releasing devices have been used extensively in cattle for many years and for many purposes, among them to prolong the luteal phase while controlling follicle development in synchronized breeding programs,[2,4,5,8,11,12,14,24,25,28,29,31] to investigate follicular development[1,6] as a treatment for cystic ovarian disease[10,30] and anestrus,[18,21,33] and as adjunct therapy to reduce pregnancy loss early in gestation.[15] Recently, researchers have used intravaginal progesterone-releasing devices to help elucidate an association between liver metabolic rate, dry matter intake and steroid metabolism.[26]

Early research focused on the PRID product, which contained 1.96 grams of progesterone in a silastic T-shaped spine.[13,14,15,25,29] More recently, researchers have utilized a number of intravaginal progesterone-releasing products in their studies, with progesterone concentrations ranging from 1.38 to 1.9 grams. Only one is

currently commercially available in the United States,[a] which contains 1.38 grams progesterone.

Intravaginal progesterone-releasing devices have been shown to increase serum progesterone concentrations in treated animals.[13,17,22,25,29,32] The mode of action of these devices has been theorized to be based on induction of dominant follicle regression through decreased LH pulse frequency in response to elevated progesterone levels in blood.[1,3,6,9]

Reuse of the PRID product containing 1.96 grams of progesterone has been shown to increase serum progesterone concentrations in dairy and beef heifers.[20,31,32] Other studies using CIDRs containing 1.9 g progesterone reported that plasma progesterone remained above 1.9 ng/mL and 2.3 ng/mL for 14 and 15 days, respectively.[17,22] One might reasonably expect that insertion of new and once-used 1.9 g CIDRs for seven days each would increase blood progesterone concentrations after each use.

It is common practice among cattle producers in the United States to reuse the 1.38 g CIDR product multiple times as a component of estrus synchronization programs. Anecdotal evidence from producers suggests that used 1.38 g CIDRs seem to work as part of an estrus synchronization protocol. Reuse of CIDRs occurs routinely, despite manufacturer's instructions that inserts should not be reused because of concern over possible transmission of venereal or blood-borne diseases.

However, despite the commonality of the reuse practice, data supporting the efficacy of reuse of the 1.38 g CIDR product is lacking. Only one peer-reviewed publication compared new versus once- and twice-used CIDRs, but the product used in the study originally contained 1.9 grams. Additionally, each animal received either estradiol, progesterone or both hormones at the time of CIDR insertion and the dependent variable was pregnancy rate.[9] The authors reported a significant decrease ($P<0.05$) in pregnancy rate in cattle that received twice used CIDRs compared to cattle that received new or once-used CIDRs, but there was no determination of serum progesterone concentrations associated with the new and reused CIDRs.

The current study was designed to determine the effect of new and reused CIDRs containing 1.38 g progesterone on serum progesterone concentrations in cows that received the same CIDR twice in succession. The hypothesis of this study was that there would be no difference in serum progesterone concentrations obtained after insertion of new and, subsequently, used CIDRs, and that both concentrations would be significantly higher than baseline serum progesterone concentrations. We expected there would be little or no decrease in the amount of progesterone released by CIDRs and absorbed by each cow between the first use and reuse.

Materials and Methods

Adult Holstein-Friesian dairy cows were studied. Over a three-week period, non-pregnant, lactating cows that had normal reproductive tracts, a corpus luteum diagnosed via palpation per rectum, and in good overall health (no mastitis, lameness, systemic illnesses) were selected for participation in the study. Cows ranged in age from two to seven years, from 44 to 285 days-in-milk and from 46 to 121 lb (21 to 55 kg) of average daily milk production.

The cows were housed at the University of Illinois Dairy in Urbana, Illinois. The study was conducted over a two-month period during late spring of 2005. Protocols for use of the university animals were approved by the University of Illinois Institutional Animal Care and Use Committee. All cows in the study were managed according to standard university protocols, and were maintained with the rest of the herd (n = 280) in either free stall or comfort stall housing. All cows in the study received a standard total mixed ration (TMR) and were milked twice daily in a 24-unit milking parlor. No changes in basic management protocols occurred during the study.

Forty cows were enrolled in three cohorts, designated Cohort 80 (eighteen cows), 81 (fifteen cows) and 82 (seven cows). Each cohort began the study one week apart, so that cows were at various stages of the study at any given time to randomize potential environmental effects, such as weather and changes in feed, on individual cow response.

Cows in each cohort followed the standard study protocol of three consecutive trials, designated T1, T2 and T3, each lasting approximately thirteen days, for a total study protocol of 40 days per cow. Prior to the start of each trial, each cow received 5.0 mL of dinoprost[b] intramuscularly, so that each cow started each of the three trials either two or three days after dinoprost administration. Subsequent serum samples for progesterone determination occurred on approximately the same day after dinoprost administration for each of the three 13-day trials.

During T1, each cow was palpated per rectum for ovarian structures, a blood sample was taken for progesterone assay to evaluate palpation accuracy for diagnosing the presence of a functional corpus luteum, and 5.0 mL of dinoprost was administered by intramuscular injection on study day zero.

On days three, six and nine (study days 3, 6, and 9), blood samples were drawn for progesterone assay. On day 13 (study day 13), a blood sample was drawn for progesterone assay, and 5.0 mL of dinoprost was administered by intramuscular injection.

During T2, a new CIDR was inserted on the morn-

ing of day five (study day 18), and removed late in the afternoon of day 12 (study day 25). Blood samples were drawn for progesterone assay on days two, five and eight (study days 15, 18 and 21). On day 12 (study day 25), in addition to CIDR removal, a blood sample was drawn for progesterone assay and 5.0 mL of dinoprost was administered by intramuscular injection.

After removal in Trial 2, each CIDR was washed with dilute chlorhexidine solution,[c] patted dry with paper towels and allowed to air dry before storing in individually identified zip-lock plastic bags.

During T3, used CIDRs were re-inserted on the morning of day five (study day 30) into the same cows from which they were removed, and removed late in the afternoon of day 12 (study day 37). Blood samples were drawn for progesterone assay on days two, five and eight (study days 27, 30 and 33). On day twelve (study day 37), in addition to CIDR removal, a blood sample was drawn for progesterone assay and 5.0 mL of dinoprost was administered by intramuscular injection.

On day 39 (study day 39), each cow was given 100 µg gonadotropin releasing hormone[d] and bred by artificial insemination 16 hours later (study day 40).

Each cow in the study had blood samples taken for progesterone assay on days two or three, five or six, eight or nine, and 12 or 13 of each of the three trials so that the results of the samples could be easily compared (Figure 1).

Blood samples were taken from the tail vein, allowed to clot in red-top tubes and centrifuged within 45 minutes of sampling. Serum was extracted, transferred to plastic sample tubes and each tube was labeled with cow ID, date of sample and cohort number. Each sample was frozen and stored until all sampling was completed. Serum was evaluated for progesterone concentration using a competitive binding, double extraction of serum with petroleum ether and subsequent ELISA, as previously reported by Rasmussen, *et al.*[23]

During Trials 1 and 3, cows were checked twice daily to determine if inserted CIDRs were still in place. Any CIDR found to be malpositioned was immediately repositioned.

Descriptive and statistical analysis was performed using SAS version 9.1 statistical software package (SAS Institute Inc., Cary, NC). Significance was set at $P<=0.05$. Sample means were calculated for progesterone concentrations from each trial and were compared between each trial. Multi-variable comparisons of mean P4 concentrations were performed for the four sample days within each trial. Least squares linear regression was used to compare mean serum progesterone levels in each trial to days open, days-in-milk, lactation number, milk weight and mature equivalent milk production at the beginning of the study.

Results

One cow was removed from the study after she lost her used CIDR during the third trial, leaving 39 cows in the study.

Thirty-seven samples showed serum progesterone concentrations greater than 1.1 ng/mL, suggestive of functional luteal tissue, as reported in previous studies.[19,27,30] Serum sample series from the two cows that had initial serum progesterone levels below 1.1 ng/mL were removed from further analysis. Baseline samples from cows remaining in the study were not included in calculation of serum progesterone means for Trial 1.

Each cow had four samples taken per trial, for a total of 12 samples per cow. The average serum proges-

Figure 1. Study protocol of three consecutive 12-day trials per cow.

							CIDR IN ↓		CIDR OUT ↓		CIDR IN ↓		CIDR OUT ↓		
	PGF ↓				PGF ↓				PGF ↓				PGF ↓	GNRH ↓	
	P4	P4	P4	P4	P4	P4	P4	P4	P4	P4	P4	P4	P4		TAI
Days	0	3	6	9	13	15	18	21	25	27	30	33	37	39	40
	|——Trial 1——|						|——Trial 2——|				|——Trial 3——|		

13 samples for P4 on days noted
PGF2a on days 0, 13, 25 and 37
CIDR IN on days 18 and 30
CIDR OUT on days 25 and 37
GnRH on day 39
TAI on day 40

terone concentrations from the three trials ranged from 2.7243 ng/mL in Trial 1, to 4.0973 ng/mL in Trial 2, to 2.7027 ng/mL in Trial 3.

Freidman's test of equal variances demonstrated that variances between sample series were not significantly different ($P<0.2897$). Samples were compared for variance using two-way ANOVA for nonparametric samples, both between serum sample series and within each sample series. Analysis of variance indicated a significant difference ($P<0.0006$) between serum progesterone levels from Trials 1, 2 and 3. Post-hoc analysis using Scheffe's test detected significant differences between Trials 1 and 2, and between Trials 2 and 3. There was no significant difference in means of serum progesterone between Trials 1 and 3 (Table 1).

Serum progesterone concentrations, segregated by day of the study, were compared across the three trials. Friedman's test for analysis of variance showed that mean serum progesterone concentrations on days two and three ($P=0.0163$), five and six ($P=0.0002$), and eight and nine ($P=0.0423$) of the study were significantly different and varied between trials. Serum progesterone concentrations from days 12 and 13 of the study were not significantly different ($P=0.2106$; Table 2 and Figure 2).

Table 1. Means of serum progesterone (ng/mL) by Trial 1, 2 or 3. Mean progesterone from Trial 2 was significantly different from Trials 1 and 3.

Trial	Mean	Standard deviation	Mean rank
1	2.7243	1.7774	1.73
2	4.0577	1.9197	2.51[a,b]
3	2.7027	1.3133	1.76

[a] Friedman two-way non-parametric ANOVA statistic = 14.952, $P<0.0006$.
[b] ANOVA F value 8.27, Scheffe's Test for differences between Trials 1 and 2 and between Trials 2 and 3; $P<0.05$.

Paired T-tests were used to compare blood sample results for differences in mean progesterone concentrations between each of the three trials. Comparison of means between sample series T1 and T2 demonstrated a statistically significant increase ($P=0.0028$) in serum progesterone consistent with the exogenous source of progesterone from the intravaginal insertion of new CIDRs. Comparison of sample series T2 and T3 indicated a statistically significant decrease ($P=0.0009$) in serum progesterone when comparing samples from cows that received a new CIDR versus the same cows receiving the used CIDRs. Comparison of sample series T1 and T3 showed no statistically significant difference ($P=0.9494$) in serum progesterone concentrations when comparing cows with no CIDRs inserted to the same cows with used CIDRs (Table 3).

Discussion

The desired effect of exogenous progesterone supplementation in estrus synchronization protocols is to increase blood progesterone concentrations above baseline concentrations adequate to suppress LH levels.

The hypothesis of the current study was that there would be no difference in progesterone concentrations in serum taken from cows following insertion of new, and subsequently, used 1.38 g CIDRs. Under the conditions of the study, serum progesterone concentrations following insertion of used CIDRs were not significantly different from baseline samples. There was a significant decrease in serum progesterone concentrations following insertion of used CIDRs when compared to progesterone concentrations associated with the use of new CIDRs. Our results suggest that there was either a significant decrease in progesterone available from the CIDR after one use, or there was a decreased absorption of progesterone available from the used CIDRs or there was induction of increased steroid metabolism by the liver after Trial 2.

We were also interested in whether production factors influenced serum progesterone. We used least

Table 2. Multi-variable comparisons of mean P4 concentrations (ng/mL) by day (2, 3), (5, 6), (8, 9), (12, 13).

Trial No.	Mean serum P4 (Rank)				
	Trial	Day 2-3	Day 5-6	Day 8-9	Day 12-13
1	2.724 (1.73)	1.646 (2.00)	1.964 (1.80)	2.739 (1.76)	4.095 (1.93)
2	4.058 (2.51)	1.544 (2.32)	4.367 (2.54)	4.274 (2.32)	6.046 (2.23)
3	2.703 (1.76)	1.051 (1.68)	1.864 (1.66)	3.508 (1.92)	4.467 (1.84)
P-value	0.0006*	0.0163*	0.0002*	0.0423*	0.2106

*Statistically significant at $\alpha = 0.05$.

squares linear regression to compare mean serum progesterone levels in each trial to days open, days-in-milk, lactation number, milk weight and mature equivalent milk production at the beginning of the study. There was no statistical association between any of these parameters and serum progesterone levels.

In addition to the lack of apparent efficacy of reused CIDRs to cause an increase in serum progesterone concentrations, we noted a couple of potential complications with reuse of CIDRs. Despite cleaning each removed CIDR with multiple cold water rinsing in a dilute chlorhexidine solution (0.0047%; 90 mL in 3.78 L of clean tap water), patting dry with a paper towel and allowing to air dry, we still noted three cases of purulent vaginitis in cows when CIDRs were reused (3 of 37, 8.1%), compared to one case of vaginitis (1 of 37, 2.7%) in the same cows the first time that they received CIDRs. Odds ratio for developing vaginitis after exposure to used CIDRs compared to new CIDRs was 3.18, with a confidence interval of 0.3149 to 32.041 (P=0.6072). Cause of the vaginitis could not be determined from the present study, but could be due to either bacterial contamination of the used, albeit washed, CIDRs or due to foreign body reaction stimulated by reuse. Vaginitis was not associated with pregnancy or open status following timed artificial insemination.

Additionally, previous research suggested that intravaginal insert loss rates can be quite high.[7,9,16] Despite cutting tails off CIDRs to minimize removal by lot mates, we noted a tendency for reused CIDRs to become malpositioned (white insert spine readily visible at the vulva). After insertion of new CIDRs, only two became misplaced and had to be repositioned (2 of 37, 5.4%). However, eight reused CIDRs had to be repositioned (8 of 37, 21.6%). Although only one CIDR was lost during the study, the odds ratio for reused CIDRs becoming malpositioned compared to new CIDRs was 4.96 (CI = 0.9779 to 25.214; P=0.0812). Had we not inspected cows twice daily for CIDR placement, both new and reused CIDRs would have been lost and the cows eliminated from the study.

We also compared the pregnancy rate (13 pregnancies out of 37 breedings (35%)) from TAI after removal of the reused CIDR and induced luteolysis to the rest of the herd pregnancy rate over the same time period (10 of 30, 33%). The odds that a cow enrolled in the present study became pregnant from the TAI that concluded the study compared to the cows that were bred via a standard ovsynch protocol over the same three-week period, was 1.0833 (CI = 0.3923 to 2.9916; P=0.9023).

The study is subject to three potential limitations: could washing CIDRs with dilute chlorhexidine solu-

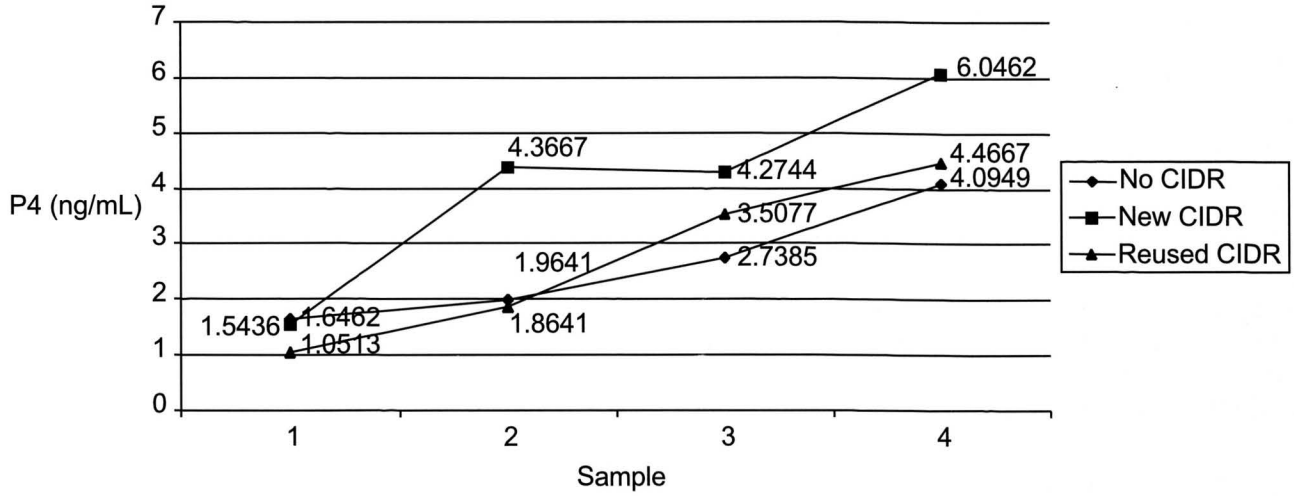

Figure 2. Comparison of serum progesterone concentrations by day of sampling within each trial.

Table 3. Paired t-test comparison of means of serum progesterone concentrations for Trials 1, 2 and 3.

Comparisons	Mean difference	95% CI	P - value
Trial 1 vs. Trial 2*	-1.3730	-2.2392 to -0.5066	0.0028
Trial 2 vs. Trial 3*	1.3946	0.3864 to 0.6109	0.0009
Trial 1 vs. Trial 3	0.0216	-0.6649 to 0.7081	0.9494

*Statistically significant at $\alpha = 0.05$.

tion leach out imbedded progesterone from each CIDR? Personal communication with the manufacturer's product support veterinarian (Lisa Halbert, DVM, PhD, Veterinary Medical Investigations and Product Support, Pfizer Animal Health, October 18, 2006) indicated that dilute chlorhexidine solution, since it contains minimal alcohol or other defatting agents, would have no effect on levels of progesterone remaining in a once-used CIDR.

Secondly, could the lack of significant rise in serum progesterone following reuse of CIDRs result from an induced refractory period for systemic absorption of progesterone? To answer that question, future work might schedule a second baseline series of progesterone samples in the study protocol to be taken between initial insertion of CIDRs and reuse.

Thirdly, could the exposure of exogenous progesterone from Trial 2 have induced increased metabolism of steroids by the liver? Such a phenomenon has been described in high-producing dairy cattle.[26] Further study might compare progesterone metabolism enzymes in cattle receiving new versus used CIDRs.

Conclusions

Results of the present study indicate that reused CIDRs originally containing 1.38 g progesterone did not increase serum progesterone concentrations in adult dairy cows as expected. If it is accepted that an increase in serum progesterone over baseline concentrations is an important outcome from the use of intravaginal progesterone inserts in synchronization protocols, then determination whether an increase in serum progesterone concentrations actually occurs is important. The outcome variable of this study was not pregnancy rate, but simply serum progesterone concentrations before and after use of new and reused 1.38 g progesterone CIDRs.

Because of the lack of effectiveness of reused 1.38 g CIDRs in increasing serum progesterone concentrations, we cannot recommend the reuse of 1.38 g CIDRs as an aid for synchronizing estrus cycles in adult dairy cows.

Acknowledgements

This study was funded solely by support from the University of Illinois, College of Veterinary Medicine. No sources of external funding were used.

We would like to thank Dr. Milo Wiltbank, University of Wisconsin-Madison, for analysis of serum progesterone, and Drs. Yvette Johnson and San Myint, University of Illinois at Urbana-Champaign, for statistical analysis.

Footnotes

[a] EAZI-BREED™ CIDR® 1.38 g, Pfizer Animal Health, Inc., Kalamazoo, MI
[b] Lutalyse®, Pfizer Animal Health, Inc., Kalamazoo, MI
[c] Nolvasan® Solution, Ft. Dodge, IA
[d] GnRH Ovacyst®, Phoenix Scientific, St. Joseph, MO

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Abstract

Precalving Fat Mobilization and Body Condition Score: Risk Factors for Left Displaced Abomasa, a Study of Five High Yielding Herds in Southwest England

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Cattle Practice (2006) 14(3):209-212

Precalving fat mobilization was investigated as a predisposing factor for left displaced abomasums (LDA) in 5 high yielding herds (>8500 litre 305 day average) in the south west of England. The herds had suffered a high LDA incidence in the previous year and blood samples were taken from the dry cows in the last week prior to calving as part of a monitoring exercise. Body condition score (BCS) was assessed at the same time. Cows with high serum concentration of non-esterified fatty acids (NEFAs) precalving were significantly more likely to subsequently suffer from LDA. Precalving BSC >3.5 was also a significant predisposing factor for the condition.