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## Dairy Research 2007

Kansas Agricultural Experiment Station

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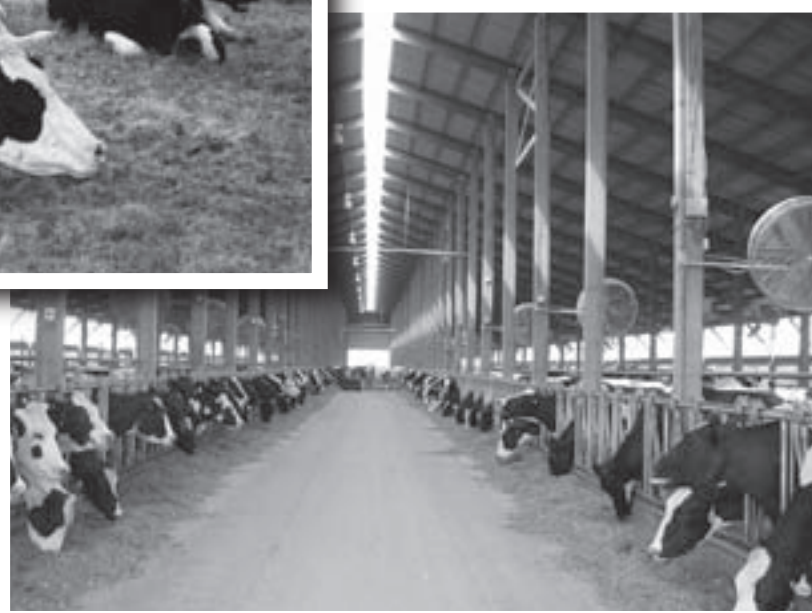
## Dairy Research 2007

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# DAIRY RESEARCH 2007



*Report of Progress 984*

**Kansas State University Agricultural Experiment Station  
and Cooperative Extension Service**

# DAIRY DAYS PROGRAM

**10:30 a.m.**

Welcome: County Agent

**10:35 a.m.**

"Factors Influencing the Use of By-Products in Lactation Rations"

*Mike Brouk*

**11:20 a.m.**

"Nutritional Strategies for a Healthy Transition to Lactation: An Update"

*Barry Bradford*

**Noon**

Lunch: Meal sponsored by the Kansas Dairy Association and the Kansas Dairy Commission

**12:45 p.m.**

Update from the Kansas Dairy Association and Voices for Choices

**1:00 p.m.**

"Heat Abatement of Older Dairy Facilities"

*Joe Harner*

**1:45 p.m.**

"Application of the Progesterone (CIDR) Insert in Artificial Insemination  
– Breeding Programs of Dairy Cattle"

*Jeff Stevenson*

**2:30 p.m.**

Adjourn

**December 11, 2007**

10:30 a.m. - 2:30 p.m.

*Valentino's Restaurant*

*Seneca, KS*

**December 13, 2007**

10:30 a.m. - 2:30 p.m.

*Whiteside Amish Community Building*

*Whiteside, KS*

## *Dairy Research 2007*

### FOREWORD

Members of the Dairy Team at Kansas State University are pleased to present the 2007 Dairy Research Report of Progress. Dairying continues to be a viable business and contributes significantly to the agricultural economy of Kansas. In 2006, dairy farms accounted for 3.3%, or \$328 million, of all Kansas farm receipts, ranking 6<sup>th</sup> among all Kansas farm commodities. In the United States, Kansas had the greatest percentage increase in milk produced between 1999 and 2004 (+57.7%). During 2002, Kansas moved into the top 10 (#8) for milk production per cow. At the end of 2006, Kansas ranked #9 (20,920 lb). Wide variation exists in the productivity per cow as indicated by the production testing program (Heart of America Dairy Herd Improvement Association [DHIA]). Nearly 111,000 cows from Kansas, Nebraska, Oklahoma, Arkansas, North Dakota, and South Dakota [including herds from Colorado (1), Iowa (27), and Missouri (16)] were enrolled in the DHI program beginning January 1, 2006. A comparison of Kansas DHIA cows with all those in the Heart of America DHIA program in 2006 is shown in the table below.

#### **Comparison of Heart of America (HOA) Cows with Kansas Cows - 2006**

Item	HOA	KS
No. of herds	669	221
No. of cows/herd	161	151
Milk, lb	19,618	20,026
Fat, lb	726	748
Protein, lb	608	627
SCC × 1,000	352	362
Calving interval, mo.	14.4	14.7

Most of this success occurs because producers better manage what is measured in monthly DHI records. Continued emphasis should be placed on

furthering the DHI program and encouraging use of its records in making management decisions. In addition, continued use of superior, proven sires and emphasis on superior genetics in artificial insemination (AI) programs is essential.

The excellent functioning of the K-State Dairy Teaching and Research Center (DTRC) results from the special dedication of our staff. We acknowledge our current DTRC staff for their contributions: Michael V. Scheffel (Manager), Daniel J. Umsheid, Alan J. Hubbard, Kris Frey, Alex Blecha, and Robert E. Fiest. Special thanks are given to Jamie Wilson, Cheryl K. Armendariz, and a host of graduate and undergraduate students for their technical assistance in our laboratories and at the DTRC.

Milk production from 238 cows at the DTRC continues to improve according to our last test day in October 2007. Our rolling herd average for milk surpassed 30,000 lb for the first time in August 2006. Our rolling herd averages for our October test were 28,561 lb of milk, 1,061 lb of fat, and 888 lb of protein.

Thorough, quality research is not only time intensive and meticulous, but also expensive. However, each dollar spent for research yields a 30 to 50% return in practical application. Those interested in supporting dairy research are encouraged to consider participation in the Livestock and Meat Industry Council (LMIC), a philanthropic organization dedicated to furthering academic and research pursuits by the Department of Animal Sciences and Industry. Additional details about the LMIC are included at the end of this report.

J. S. Stevenson, Editor  
2007 Dairy Research Report of Progress

*Dairy Research 2007*

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*Dairy Research 2007*

## **NUTRITIONAL STRATEGIES FOR A HEALTHY TRANSITION TO LACTATION: AN UPDATE**

*Barry Bradford*

### **Summary**

Reducing cull rates and improving fertility in early lactation has a dramatic effect on the profitability of many dairies. Continuing research on transition cow nutrition has led to the development of an array of nutritional strategies to prevent disorders during the transition period. It is important, however, to realize that some of these strategies have similar modes of action, and as such, their effects are not likely to be additive. Producers should work with their nutritionist and veterinarian to identify the most prevalent transition problems in the herd and review options for preventing those disorders.

(Key words: transition period, monensin, choline, propylene glycol, vitamin E.)

### **Introduction**

Despite significant advances in nutritional management of dry cows in recent years, the transition period remains the most challenging component of the production cycle for dairy cattle. Disorders such as mastitis, metritis, displaced abomasum, ketosis, and fatty liver are common during the transition period, often resulting in high cull rates and contributing to poor fertility in early lactation. The following is a brief update on the latest strategies being employed to improve the health of transition dairy cows.

### **Energy Density and Forage Quality**

Excessive body condition at calving is clearly a major risk factor for metabolic disorders during the transition period, and all dairies should focus on limiting body condition gain during late lactation and in far-off dry cows (21 to 60 days before calving). Substantial disagreement, however, remains on the question of whether “steam-up” rations are necessary for close-up cows (the final 21 days before calving).

The logic behind the steam-up approach is simple. Immediately switching a cow from a forage-based ration to a high-concentrate ration can cause problems, particularly acidosis and variable feed intake. The steam-up ration is intended to provide time for the rumen to adapt to a higher-grain diet before lactation begins. In contrast, research at the University of Illinois has demonstrated that low-energy diets incorporating wheat straw can be fed throughout the dry period without causing problems during the transition to lactation. These rations are formulated for 40 to 50% forage neutral detergent fiber (from corn silage, straw, and alfalfa hay) and have  $NE_L$  values of about 0.62 Mcal/lb of dry matter. Based on these studies, some nutritionists have concluded that a steam-up ration is unnecessary, allowing formulation of a single ration for all dry cows.

On the other hand, numerous studies have supported the idea that steam-up rations can improve metabolic health in early lactation. Two of the most complete studies compared rations providing approximately 0.59 versus 0.74 Mcal NE<sub>L</sub>/lb of dry matter, primarily by altering fiber and starch concentrations. Both of these studies found that the higher-energy, close-up rations increased feed intake and energy balance after calving. As a result, steam-up rations limited the increase in plasma non-esterified fatty acids (NEFA) and liver triglycerides in early lactation, which is expected to decrease the incidence of fatty liver, ketosis, and possibly other disorders.

How should we reconcile these findings? It may be that each approach provides a distinct benefit for transition cows. Incorporating a slowly-digested fiber source (such as wheat straw) in the close-up ration maintains ruminal fill during short periods of reduced feed intake, helping to prevent displaced abomasum and reducing risk of acidosis when cows begin eating again. In contrast, higher-energy diets provide an opportunity to adapt ruminal microbes to an increase in digestible carbohydrates before the lactation ration is introduced. Perhaps more importantly, steam-up rations typically increase propionate production, providing more glucose precursors to the liver as lactation begins.

The ideal close-up ration may incorporate a slowly-digested fiber source within a relatively high-energy ration. Proportions of neutral detergent fiber and starch in close-up rations can be close to those of a lactation ration, with perhaps 1/3 of the dietary forage replaced with an ingredient such as wheat straw or prairie hay. Feeding a low-energy ration to far-off cows followed by this type of steam-up ration for the final 21 days of the dry period may minimize the incidence of transition cow disorders. Alternatively, facilities and labor situations on certain farms may

make a single-group dry cow program a better option. In either case, producers need to ensure cows are actually consuming the ration that is being fed. Ingredients such as wheat straw typically need to be chopped to reduce particle size to 2 inches or less, and the ration must be moist enough to prevent cows from sorting.

## Choline

Availability of a rumen-protected choline (RPC) product has spurred a great deal of interest in the potential for choline to prevent fatty liver in transition cows. Choline is an essential component of the machinery responsible for the exporting triglycerides from the liver, and if a lack of choline availability limits this process, then RPC may prevent triglyceride accumulation in the liver.

Recent studies have strengthened the evidence that RPC can be an effective tool to limit fatty liver. Feeding RPC slowed the rate of liver triglyceride accumulation in feed-restricted cows and also increased the rate of triglyceride clearance from the liver in cows recovering from feed restriction. Many more experiments have demonstrated a positive effect of RPC on milk production in early lactation. One recent study of 182 transition cows found that RPC increased milk production by 2.6 lb/day during the first 60 days of lactation. Interestingly, this production response was entirely due to a 9.7 lb/day response to RPC in overweight cows (body condition score  $\geq 4.0$ ), with no production response among cows with body condition scores  $< 4.0$ . This dramatic benefit among the overconditioned cows underscores the importance of solving metabolic problems to improve productivity.

Based on the strength of these new studies, herds with overconditioned cows, or those having a large incidence of ketosis, should consider utilizing RPC in their transition diets.



Most studies with RPC have supplemented cows at about 60 grams per cow/day during the close-up period and through the first 3 to 4 weeks of lactation.

### **Glucose Precursors**

Feeding or drenching glucose precursors remains a popular approach to ketosis prevention. Given the cost of supplements such as propylene glycol (PG) and calcium propionate, however, the decision to use these nutrients should be made carefully.

Studies have consistently shown that drenching cows with PG has positive effects on metabolic health. Drenching with PG provides the cow with compounds that can be used for glucose synthesis, resulting in increased plasma concentrations of glucose and insulin. This, in turn, decreases plasma NEFA and ketone concentrations. Because drenching transition cows is a labor-intensive process that is impractical on many farms, some dairy producers simply incorporate PG into fresh cow rations. Unfortunately, there is little evidence that dietary PG provides the same benefits as drenching with PG. The problem with delivering PG in the diet is that ruminal microbes are capable of metabolizing PG, and if small amounts of PG are consumed throughout the day, most of it is degraded before it can be absorbed. This problem is avoided with oral drenches because a large amount is delivered at once, allowing the majority of the PG to be absorbed before it is metabolized in the rumen.

Calcium propionate has been evaluated as another potential glucogenic ingredient, and it remains stable when included in the ration. Few experiments with calcium propionate, however, have shown any benefit for metabolic health, and no production responses have been observed. Although propionate is an important glucose precursor in dairy cows, die-

tary calcium propionate has only an incremental effect on total propionate absorption by the cow. Furthermore, excessive propionate uptake is known to suppress feed intake. Therefore, inclusion of calcium propionate in transition cow diets is not warranted.

For farms with ketosis problems, appropriate facilities, and sufficient labor, drenching cows with PG several times in the first week of lactation may be beneficial. One benefit of the drenching protocol is that individual cows can be targeted for treatment rather than treating all fresh cows. Cows with excessive body condition, or those affected by disorders such as displaced abomasum or retained placenta, are logical candidates. It is recommended that each cow targeted for treatment be drenched with 500 cc of PG for at least 2 days.

### **Monensin**

Monensin was approved for use in lactating dairy cows several years ago. Although the only approved market claim for monensin is that it increases feed efficiency, many dairies are interested in its potential effects on energy status of transition cows.

The evidence for a beneficial effect of monensin on transition cow health is substantial. The most convincing study included 1,317 cows on 45 farms, in which cows randomly assigned to monensin treatment were administered a controlled-release capsule (CRC) beginning 2 to 4 weeks prior to calving. In this study, CRC administration decreased the collective incidence rate of retained placenta, displaced abomasum, and clinical ketosis by 30%.

Unfortunately, monensin is not available in the CRC form in the U.S. The question, then, is whether incorporating monensin in close-up or fresh cow rations can provide the same benefit. In particular, concerns exist that

the decrease in feed intake that commonly occurs during the week of calving may limit monensin intake enough to decrease its beneficial effects.

One published study has compared transition cow responses to monensin delivered in the diet versus CRC's. Although only the CRC significantly improved body condition in early lactation, dietary monensin was just as effective as the CRC at decreasing plasma ketone concentrations. Taking into account past studies demonstrating that dietary monensin can decrease plasma NEFA and ketones in early lactation and decrease time to first service, including monensin in transition cow rations likely provides at least some of the benefits observed in CRC-treated cows. Dairies wishing to incorporate monensin into transition cow diets should do so at the rate of 22 mg/kg of dry matter.

### **Antioxidants**

In the 1990s, a number of reports documented the ability of supplemental vitamin E and selenium to decrease the incidence of mastitis in early lactation. Recently, a meta-analysis showed that supplemental vitamin E

is also effective at preventing retained placenta. Several studies have also associated low plasma vitamin E concentrations with increased incidence of fatty liver and displaced abomasum. Therefore, it is recommended that transition cows be fed at least 1,500 IU of vitamin E per day and that dry and fresh cow rations include 0.3 ppm of selenium.

### **Future Research**

Several other ingredients are attracting renewed interest for their potential benefits in transition cows. Glycerol is becoming more available as the biodiesel industry grows and is often the lowest-cost glucose precursor available. Initial experiments, however, evaluating the effects of glycerol on transition cows have not demonstrated any health or production benefits. Encapsulated niacin is another product with promise for preventing fatty liver and ketosis. Niacin that survives the rumen clearly limits NEFA release from adipose tissue, but no reports exist that have evaluated effects of encapsulated niacin on transition cows. In the next several years, new research on both glycerol and encapsulated niacin should provide more insight into the efficacy of these ingredients.

*Dairy Research 2007*

## **APPLICATION OF THE PROGESTERONE (CIDR) INSERT IN ARTIFICIAL INSEMINATION PROGRAMS OF DAIRY CATTLE**

*J. S. Stevenson*

### **Summary**

Use of progesterone inserts (controlled interval drug release, CIDR) offers another option for synchronizing estrus and ovulation in replacement heifers and lactating dairy cows. Results indicate that heifers may be inseminated after detected estrus, at a fixed time (timed AI), or a combination of both. Conception rates exceed 50% in both scenarios. Practical applications of the CIDR in lactating cows have been used to resynchronize the return estrus of previously inseminated cows and as part of first-service AI-breeding protocols. Use for resynchronization has no drawbacks in previously inseminated cows, but may increase embryo survival during the first 30- to 60-days of pregnancy. No increase in the heat-detection rates of open cows is generally achieved, and no differences in return conception rates are observed between treated and control cows. First-service applications of the progesterone insert have resulted in some positive and some negative effects on timed AI (TAI) conception rates. More research is warranted to determine if an identifiable subpopulation of cows can benefit from exposure of the progesterone insert before first AI.

(Key words: heifers, cows, CIDR, Ovsynch, pregnancy rates.)

### **Introduction**

Because fertility of lactating dairy cows is poor and has decreased more than 50% since 1970, improving fertility of lactating dairy cows is economically important to dairy pro-

ducers. A negative relationship exists between dry matter intake and circulating concentrations of progesterone in lactating dairy cows. Lactating dairy cows have serum concentrations of progesterone less than those in heifers. Progesterone is important to fertility, as demonstrated by a positive correlation between serum progesterone before AI and subsequent conception rate. Progesterone, in addition to use of gonadotropin releasing hormone (GnRH) and prostaglandin F<sub>2α</sub> (PGF<sub>2α</sub>), can be used to facilitate AI by synchronizing estrus and ovulation in heifers and lactating cows. Understanding the pharmacological impact of progesterone supplementation on conception rates may lead to a better understanding of the physiological reasons for reduced fertility of lactating dairy cows.

Today, progesterone is applied in the form of an intravaginally placed insert containing 1.38 g of progesterone and is marketed as an EAZI-BREED CIDR. This is the only progesterone product approved by the U.S. Food and Drug Administration for use in cattle. Its label indications include synchronization of estrus in suckled beef cows and replacement beef and dairy heifers, advancement of first postpartum estrus in suckled beef cows, and advancement of first pubertal estrus in beef heifers. For these applications, the insert is left in place for 7 days, with an injection of Lutalyse 1 day before insert removal. Signs of estrus are then observed on days 1 to 3 after insert removal, and insemination occurs about 12 hours after the first signs of estrus.

Label indications for lactating dairy cows include synchronization of the return estrus for cows previously inseminated. The insert is applied  $14 \pm 1$  days after insemination and the insert is removed 7 days later. Again, signs of estrus are observed on days 1 to 3 after insert removal, and insemination should occur about 12 hours after detection of estrus.

The objective of this review is to provide a summary of several proven applications of progesterone in successful AI-breeding programs. Most of the programs discussed are considered to be extra label, however, their application goal is to synchronize estrus and ovulation before insemination, either based on signs of estrus or by appointment (fixed-time AI or TAI).

### **Replacement Heifers**

Injecting  $\text{PGF}_{2\alpha}$  at the end of a short-duration (7 days) CIDR treatment allows estrus in nearly all females to be synchronized during a shorter treatment period. Administering progesterone for approximately 7 days allows any female in estrus or metestrus at the outset to advance to the luteal phase before  $\text{PGF}_{2\alpha}$  is administered. Expression of estrus is prevented in the remaining females that are in proestrus or late diestrus by the progesterone insert, and those in the luteal phase respond to the injection of  $\text{PGF}_{2\alpha}$ . Therefore, the combination of both hormones shortens the overall period of treatment and generally allows normal fertility.

The standard labeled indication protocol is illustrated in Figure 1 (Option A) in which heifers are inseminated after detection of estrus. Average occurrence of estrus is about  $43 \pm 3$  hours after insert removal, if  $\text{PGF}_{2\alpha}$  is administered 24 hours before insert removal, or  $49 \pm 1$  hours if the injection occurs at insert removal. Conception and pregnancy rates are shown for Options B and C. In Option B, heif-

ers were treated at 3 locations in Kansas. In Option C, heifers were treated at 12 locations in the 6 Midwest states. Combining heat detection to 84 hours after insert removal and a cleanup TAI at 84 hours resulted in 85% of the inseminations made before the cleanup TAI. The latter two treatments administered one TAI at 60 hours. Note that measures of fertility are quite similar and administering GnRH upfront resulted in very little increase in fertility. The most consistent results at all 12 locations in Option C, however, was the last treatment, in which GnRH was administered upfront with the insert and one TAI was administered at 60 hours after insert removal.

### **Lactating Dairy Cows**

#### ***Synchronizing Return to Estrus***

Three studies have been conducted in which the CIDR insert was used to resynchronize return to estrus in lactating dairy cows (Table 1). Study 1 was conducted in 2 Kansas herds. The CIDR was inserted for 7 days beginning at day 13 after TAI (first service, Figure 2). Cows were observed for signs of estrus and re-inseminated between 20 and 26 days after TAI. Study 2 was conducted in 7 herds located in 5 states. These cows were treated with a single injection of Lutalyse and inseminated. Beginning  $14 \pm$  days after AI, the progesterone insert was placed intravaginally for 7 days. Cows were observed for estrus and inseminated between 21 and 25 days after AI. Study 3 was conducted in California in 1 commercial dairy herd. Treatment with the CIDR insert occurred at 14 days after TAI (first service).

Results of the 3 studies are found in Table 1. In 1 of 3 studies, conception rate of cows that became pregnant before treatment was slightly reduced, but in 2 studies, pregnancy loss of cows that became pregnant before treatment was reduced in response to the progesterone treatment during the third week of

pregnancy ( $14 \pm 1$  to  $21 \pm 1$  days). Heat-detection return rates were increased in study 2; but overall, the percentage of cows returning to estrus for re-insemination at the first eligible estrus did not differ from controls. Conception rate of the returned estrus was reduced in study 1, but overall, no differences were detected between treatments.

These studies clearly show that no harm is done to cows of unknown pregnancy status when treated with the progesterone insert  $14 \pm 1$  days after insemination. Return heat-detection rates are neither improved, nor are return conception rates reduced. Embryo survival seemed to be improved in pregnant cows treated with the progesterone insert. These studies illustrate that 34 to 38% of cows return to estrus after  $21 \pm 1$  days from previous AI. Emphasis on heat detection during this period is important to reducing inter-insemination intervals in cows.

### ***Use Before First Services***

Seven studies have been conducted in which the progesterone insert was incorporated into the Ovsynch or Presynch + Ovsynch protocols for lactating dairy cows receiving their first AI after calving (Figure 2). Conception rates are reported for each of these studies in Table 2. In 4 studies in which the comparison was Ovsynch vs. Ovsynch + CIDR, 3 studies (studies 1, 3, and 6) reported greater TAI conception rates in cows receiving progesterone. Conception rates, however, for older cows of study 3 and for all cows in study 2, to which the CIDR insert was applied, were less than those in nontreated cows.

In 4 studies in which the comparison was Presynch + Ovsynch vs. Presynch + Ovsynch + CIDR, positive results were reported in 2 studies (studies 4 and 7; Table 2). For cows treated with the progesterone insert (studies 2 and 5), TAI conception rates were less than in cows not treated. These obvious inconsisten-

cies are further compounded by the fact that in studies in which multiple herds were treated (e.g., study 6), a treatment  $\times$  herd interaction was detected. The interaction indicates that the treatment (addition of progesterone) was only positive in some herds.

Closer examination of 2 of the studies cited previously (studies 6 and 7), indicates that progesterone is beneficial in a subpopulation of all cows treated (Table 3). Based on blood samples collected before treatments were applied, the cycling status of cows in these 2 studies was determined. When cows had been exposed to elevated concentrations of progesterone ( $\geq 1$  ng/mL) in either 2 or 3 samples collected during 10 or 28 days before treatment, cows were classified as cycling, whereas those having consistently low ( $< 1$  ng/mL) concentrations during the same period were classified as noncycling. Table 3 illustrates these 2 classifications of cows, plus whether they had elevated concentrations of progesterone when the progesterone insert was removed and PGF<sub>2 $\alpha$</sub>  was injected before TAI.

Noncycling cows having elevated progesterone at insert removal in study 1 were known to have functional luteal tissue, because the insert was removed at least 1 hour before blood was collected to measure progesterone. In study 2, blood was collected at insert removal. For noncycling cows in study 1 with elevated progesterone before PGF<sub>2 $\alpha$</sub>  was injected, a follicle must have ovulated in response to the first GnRH injection of the Ovsynch protocol and formed a corpus luteum (CL). In these cows, the CIDR insert seemed to provide no benefit (Table 3). In contrast, noncycling cows having low progesterone before PGF<sub>2 $\alpha$</sub>  was injected remained anovulatory or noncycling and may have benefited from exposure to the insert in study 1, because TAI conception rates were three-fold greater than in no-CIDR cows (Table 3). Caution in interpreting a benefit is suggested because of the

small numbers of cows in that classification (noncycling + low concentrations). Further, this benefit was not observed for similarly treated cows in study 2, nor in another study in which TAI conception rates were 18% for 38 CIDR-treated cows and 19% for 32 nontreated cows.

Cycling cows having elevated concentrations of progesterone before PGF<sub>2α</sub> was injected were those having a functional CL at insert removal, whereas those having low concentrations at insert removal had no functional CL (likely regressed during progesterone treatment). Cycling cows, particularly those cows in which no functional luteal tissue was present at insert removal, exposed to the progesterone insert during 7 days before PGF<sub>2α</sub> was injected had greater TAI conception rates. In this case, the progesterone insert prevented premature estrus and ovulation. For comparison purposes, study 2 included cows having a CL at the onset of the Ovsynch protocol and not treated with a CIDR insert. Their TAI conception rates are shown in Table 3.

At present, it is difficult to make any recommendation about how the CIDR insert might be used to improve TAI conception rates in lactating dairy cows. Because of its cost (approximately \$10), the insert should not be used without sound evidence for its benefit

to fertility and a return on its investment to dairy producers.

One recently published study applied a “cherry picking” approach to use of the CIDR insert. This study was conducted in a dry lot dairy. Cows were treated with the Presynch + Ovsynch protocol. Presynch PGF<sub>2α</sub> injections were administered at 47 and 61 days in milk and cows detected in estrus were inseminated after *either* Presynch injection. Heat detection allowed 77% (3,974 of 5,162) of the cows to be inseminated during 33 days that followed the 2 Presynch injections until Ovsynch (n = 589) or Ovsynch + CIDR (n = 586) was applied to the remaining noninseminated cows. Those remaining cows exposed to the insert had greater TAI conception rates than nontreated cows (31 vs. 23%). Further, cows exposed to progesterone had greater concentrations of progesterone 14 days after TAI than nontreated cows, regardless of pregnancy status. Results of this study indicate that a population of cows may be responsive to the benefit of progesterone treatment. This population of cows may represent a larger proportion of noncycling cows and those which poorly express estrus. Another large-scale multi-herd study is currently being conducted to verify the results of the previous study.

**Table 1. Responses of Lactating Cows to CIDR Insert to Synchronize Return of Estrus**

Study	Treatment	Previous AI			Return AI		
		No. of cows	Conception rate, %	Pregnancy loss, %	No. of cows	Return rate, %	Conception rate, %
1	CIDR	297	42	34*	169	31	20*
	Control	327	38	56	189	27	32
2	CIDR	881	33*	...	589	34*	27
	Control	863	37	...	544	19	31
3	CIDR	373	36	16*	227	55	31
	Control	602	32	25	387	59	27
Total	CIDR	1,551	35	25**	985	38	27
	Control	1,792	36	33	1,120	34	30

\*Differs ( $P < 0.05$ ) from control within study.

\*\*Differs ( $P < 0.05$ ) from control across all studies.

**Table 2. Synchronization of Ovulation in Lactating Dairy Cows after Ovsynch or Presynch + Ovsynch Compared with Similar Treatments that Included a Progesterone Insert (CIDR)**

Study	Days since timed AI	Ovsynch		Presynch + Ovsynch	
		No CIDR	CIDR	No CIDR	CIDR
		----- No./no. (%) -----			
1	29 <sup>1</sup>	33/91 (36)	54/91 (59*)		
	57 <sup>1</sup>	18/91 (20)	41/91 (45*)		
2	29 <sup>1</sup>	66/154 (43)	48/150 (32)	76/157 (48)	69/153 (45)
3	40 to 45				
	1 <sup>st</sup> lactation	18/90 (20)	34/89 (38*)		
	≥ 2 <sup>nd</sup> lactation	44/160 (28)	38/166 (22)		
4	40-45			154/415 (37)	178/414 (43*)
5	27			132/338 (39)	121/335 (36)
6	28	130/321 (40)	159/313 (51*)		
	56	102/321 (32)	130/313 (42*)		
7	33			28/116 (24)	50/155 (32*)
	61			24/116 (21)	45/155 (29*)
Totals	29 to 45	291/816 (36)	335/809 (41*)	390/1,026 (38)	418/1,057 (40)
	56 to 61	120/412 (29)	171/404 (42*)	...	...

\*Differs ( $P < 0.05$ ) from no-CIDR cows.

<sup>1</sup>Greater ( $P < 0.05$ ) conception rates for Presynch cows. No effect of CIDR insert.

**Table 3. Pregnancy Rates at Day 56-61 After Timed Insemination According to Luteal Activity before Treatment and Concentrations of Progesterone at the Time of PGF<sub>2α</sub> Injection**

Pretreatment cycling status <sup>1</sup>	Study	Serum progesterone before PGF <sub>2α</sub> <sup>2</sup>	No CIDR		CIDR		CL present <sup>3</sup>	
			No CIDR		CIDR			
			-----		No./no. (%) -----			
Noncycling	1	High	19/58 (33)	17/47 (36)			...	
	2	High	8/34 (24)	16/56 (29)			7/25 (28)	
			Subtotal high	27/92 (29)	33/103 (32)			
	1	Low	4/38 (11)	15/41 (37)			...	
	2	Low	2/17 (12)	1/30 (3)			2/12 (17)	
			Subtotal low	6/55 (11)	16/71 (23†)			
			Noncycling total	33/147 (22)	49/174 (28)			
Cycling	1	High	69/178 (39)	79/179 (44)			...	
	2	High	14/54 (26)	24/59 (41)			236/651 (36)	
			Subtotal high	83/232 (36)	103/238 (43†)			
	1	Low	10/47 (21)	19/46 (41)			...	
	2	Low	0/11 (0)	4/10 (40)			28/108 (26)	
			Subtotal low	10/58 (17)	23/56 (41*)			
			Cycling total	93/290 (32)	126/294 (43*)			

\*Differs ( $P < 0.05$ ) from No CIDR cows.

†Differs ( $P < 0.10$ ) from No CIDR cows.

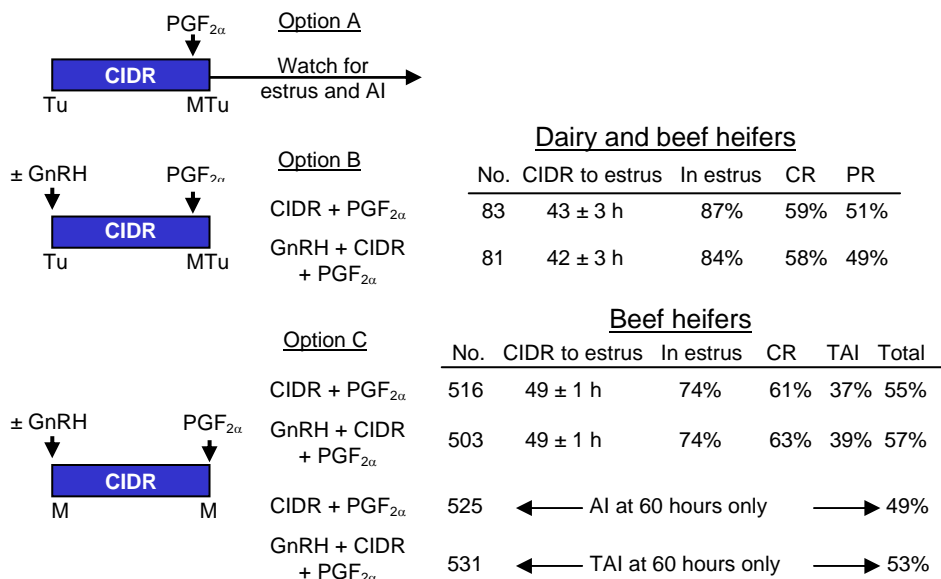
<sup>1</sup> Based on progesterone concentrations measured in 3 blood serum samples collected before each Presynch PGF<sub>2α</sub> injection and before the first GnRH injection of the Ovsynch protocol.

<sup>2</sup> Low = concentrations of progesterone  $< 1$  ng/mL, and high =  $\geq 1$  ng/mL.

<sup>3</sup> Presence of a corpus luteum assessed by transrectal ultrasonography at the first GnRH injection of the Ovsynch protocol. Some CIDR-treated cows may have had elevated progesterone because of the CIDR insert.

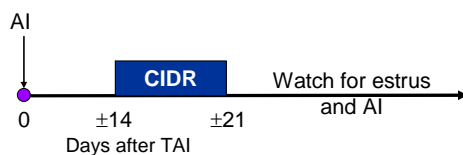


## Replacement Heifer AI-Breeding Options

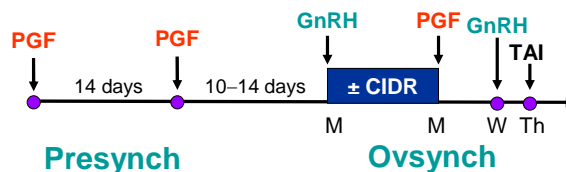


**Figure 1. Various AI Breeding Protocols for Heifers.** Option A is the labeled indication in which PGF<sub>2α</sub> is administered 24 hours before insert removal, and all inseminations are made after detected estrus. Option B tested at 3 locations included or did not include an upfront GnRH injection (CIDR insert to estrus in hours; CR = conception rate; PR = pregnancy rate; Total = combined pregnancy rate for CR + TAI). Option C tested at 12 locations includes insemination after detected estrus (CR) plus a cleanup TAI at 84 hours after insert removal. The last two protocols in Option C are TAI options with no heat detection. AI = artificial insemination; TAI = timed AI; M = Monday; Tu = Tuesday; GnRH = gonadotropin releasing hormone; PGF<sub>2α</sub> = prostaglandin F<sub>2α</sub>; and CIDR = progesterone insert.

### Use of CIDR Insert to Resynchronize Return Estrus



### Use of the CIDR Insert before First AI



**Figure 2. Application of the Progesterone (CIDR) Insert in Lactating Dairy Cows to Resynchronize the Return to Estrus in Cows of Unknown Pregnancy Status or as Part of a First-service AI Breeding Protocol.** AI = artificial insemination; PGF = prostaglandin F<sub>2α</sub>; GnRA = fonadotropin releasing hormone; TAI = timed AI; M = Monday; W = Wednesday; Th = Thursday.

*Dairy Research 2007*

## **DETECTION OF NONCYCLING COWS BY HEATMOUNT DETECTORS AND ULTRASOUND BEFORE TREATMENT WITH PROGESTERONE**

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### **Summary**

Our objective was to determine accuracy of identifying anovulatory lactating dairy cows before the application of a timed AI protocol [with or without progesterone supplementation via a controlled internal drug release (CIDR) insert and 2 different timings of AI] by using heatmount detectors and a single ovarian ultrasound examination. At 6 Midwest locations, 1,072 cows were enrolled in a Presynch protocol (2 injections of prostaglandin F<sub>2α</sub> (PGF<sub>2α</sub>) 14 days apart) with the second injection administered 14 days before initiating the Ovsynch protocol (injection of gonadotropin releasing hormone (GnRH) 7 days before and 48 hours after PGF<sub>2α</sub> injection, with timed AI at 0 or 24 hours after the second GnRH injection). Heatmount detectors were applied to cows at the time of the first Presynch injection, assessed 14 days later at the second Presynch injection and again at initiation of the Ovsynch protocol, and ovaries were examined for presence of a visible corpus luteum (CL) by ultrasound before initiation of treatment. Treatments were assigned to cows based on presence or absence of a visible CL: 1) anovulatory (no CL + CIDR insert for 7 d); 2) anovulatory (no CL + no CIDR); and 3) cycling (CL present). Further, every other cow in the 3 treatments was assigned to

be inseminated concurrent with the second GnRH injection of Ovsynch (0 hour) or 24 hours later. Pregnancy was diagnosed at 33 and 61 days after the second GnRH injection. Heatmount detectors and a single ultrasound examination both underestimated proportions of cows classified as anovulatory or having no prior luteal activity compared with those classifications determined by concentrations of progesterone in blood serum. Overall accuracy of heatmount detectors and ultrasound was 71 and 84%, respectively. Application of progesterone to cows without a CL at the time of the first injection of GnRH reduced incidence of ovulation but improved pregnancy rates at day 33 or 61 compared with non-treated cows without a CL at the onset of the Ovsynch protocol. Pregnancy rates and pregnancy survival did not differ for cows having a CL before treatment compared with those not having a CL but treated with progesterone. Pregnancy rates were 1.5-fold greater for cows ovulating in response to the first GnRH injection. Timing of AI at 0 or 24 hours after the second GnRH injection did not alter pregnancy rates, but cows having prior luteal activity before treatment had improved pregnancy rates compared with anovulatory cows. We conclude that identifying anovulatory cows by ultrasound was more accurate than by heatmount detectors. Subsequent treatment of

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<sup>1</sup>Authors and their locations: Krisher (IN) Lamb, Larson, and Dahlen (MN), Pursley and Bello (MI), Fricke, Wiltbank, and Brusveen (WI), and Burkhart, Youngquist, and Garverick (MO).

potential anovulatory cows with progesterone failed to improve fertility but had benefit for cows with prior estrous cycles at the onset of the timed AI (TAI) protocol, regardless of luteal status before the final luteolytic injection of PGF<sub>2α</sub>.

(Key words: anovulation, controlled internal drug release, Ovsynch, pregnancy rates.)

## Introduction

Because fertility of lactating dairy cows is poor and has decreased more than 50% since 1970, improving fertility of lactating dairy cows is economically important to dairy producers. A previous study examined effects of supplemental progesterone via a progesterone-releasing, intravaginal CIDR insert in various experimental designs. Conception rates were greater for cows treated with progesterone during the Ovsynch protocol (injection of GnRH 7 days before and 48 hours after PGF<sub>2α</sub> injection, with TAI between 0 and 24 hours after the second GnRH injection) at 28 and 56 days after TAI, respectively. In that study, conception rates were more positive for both cycling and noncycling or anovulatory cows treated with the CIDR insert compared with no CIDR treatment but only at 4 of the 6 locations at 28 days and at 3 of 6 locations at 56 days after TAI. This inconsistent response is corroborated by other large-scale studies.

Estrus-detection aids including tail paint, chalk, and heatmount detectors are inexpensive tools that may aid in the detection of non-cycling cows before first AI. Likewise, examination of ovaries by transrectal palpation or ultrasonography is a means of identifying anovulatory cows that may benefit from progesterone supplementation as part of a TAI protocol.

Pregnancy rates are maximized when the TAI of the Ovsynch protocol is administered

at 16 hours after the second GnRH injection. In practice, this timing is somewhat inconvenient and does not correspond to other management activities (e.g., AI, pregnancy diagnosis, vaccinations, and other treatments) that often occur while dairy cows are locked up at the feed line after morning milkings. Further, AI-pregnancy rates were not too different when AI occurred concurrent with the second GnRH injection or 24 hours later.

The objectives of the present experiment were to determine: 1) accuracy of detecting anovulatory cows (no prior estrous cycles) by using heatmount detectors applied to cows during the 28 days before initiating the Ovsynch protocol or by a single ovarian examination employing transrectal ultrasonography at the onset of the Ovsynch protocol; 2) benefit of applying progesterone (via a CIDR insert) during the first 7 days of the Ovsynch protocol; and 3) pregnancy rates after timing of AI occurred concurrent with the second GnRH injection of the Ovsynch protocol or 24 hours later.

## Procedures

### *Experimental Locations*

This study was a collaborative project of the North Central Regional Research Project 1006 of the Cooperative States Research, Education, and Extension Service (CSREES). Similar treatments were applied to lactating Holstein cows at 6 locations (Indiana, Kansas, Michigan, Minnesota, Missouri, and Wisconsin) where co-authors were located. A total of 1,072 cows were enrolled between April 2003 and October 2005. A similar experimental design was used at each location. New cows were enrolled weekly or biweekly into breeding clusters.

### ***Experimental Protocol***

Sampling, procedures, and design of treatments are illustrated (Figure 1). Cows were enrolled in a Presynch + Ovsynch protocol. Two, 25-mg injections of PGF<sub>2α</sub> (5 mL of Lutalyse, Pfizer, New York, NY or 5 mL of Prostamate, Phoenix Scientific, Inc., St. Joseph, MO) were given i.m. 14 days apart, and the Ovsynch protocol was initiated 14 days after the second Presynch injection with the first injection of GnRH. Prostaglandin F<sub>2α</sub> (25 mg) was administered 7 days later and followed in 48 hours by a second injection of GnRH. All injections of GnRH (100 μg) were administered i.m., consisting of 2 mL of Ova-Cyst (Phoenix Scientific, Inc.).

Heatmount detectors (Kamar Inc., Steamboat Springs, CO) were affixed midline to the rump of each cow between the tail head and the tuber coxae (hook bones). Detectors were placed on all cows before the first Presynch injection and were assessed before the second Presynch and the first GnRH injection of Ovsynch. Condition of heatmount detector was recorded as red, absent, white, or partially red (leaky). A new detector was applied before each injection if the detector was activated or absent. For purposes of this experiment, sexual behavior associated with estrus was assumed to have occurred when the heatmount detector was activated or absent (ovulatory or cycling cows). Leaky or nonactivated detectors were assumed to be associated with no prior estrual activity (anovulatory or non-cycling cows).

### ***Treatments***

At the onset of the Ovsynch protocol, ovaries were examined by using transrectal ultrasonography. Ultrasonography was conducted by using a transrectal 5.0 or 7.5 MHz linear-array transducer (Aloka 500V; Corometrics Medical Systems, Inc., Wallingford, CT). Follicles and luteal structures were mapped, and follicles were sized by using internal calipers.

Treatments were assigned to cows based on presence of a visible CL (CL absent vs. CL present): 1) anovulatory (no CL + CIDR insert for 7 d); 2) anovulatory (no CL + no CIDR); and 3) cycling (CL present). Further, every other cow within the 3 treatments was assigned to be inseminated concurrent with the second GnRH injection of Ovsynch (0 hour) or 24 hours later. Pregnancy was diagnosed at 33 and 61 days after the second GnRH injection. Presence of fluid in the uterus, a CL, and a viable embryo were evidence of pregnancy. For cows that were pregnant at the first diagnosis (day 33), a subsequent ultrasound examination at day 61 was used to determine pregnancy loss.

At 3 locations (KS, MI, and MI), follicles also were mapped and sized at further ovarian exams performed by ultrasound before the PGF<sub>2α</sub> and second GnRH injections of Ovsynch and at 5 days after the second GnRH injection to determine if ovulation occurred in response to the second GnRH injection. Blood was collected at all locations before each hormonal injection and again at 5 and 12 days after the second GnRH injection. Concentrations of progesterone were measured to determine prior luteal activity before and after treatments.

## **Results and Discussion**

### ***Location Characteristics***

Various outcomes of lactating dairy cows treated at each of 6 locations are summarized in Table 1. A total of 1,072 cows were enrolled in the study in 6 Midwest states. Nearly all traits in Table 1 differed among locations, except for pregnancy rate at day 33. Calving difficulty scores averaged  $1.3 \pm 0.48$  (SD) and ranged from 1.2 to 1.7. Average body condition score after calving and before the first Presynch injection ( $37 \pm 7$  DIM) were  $2.8 \pm 0.48$  (SD) and  $2.6 \pm 0.44$  (SD), respectively.

Days in milk (DIM) at TAI averaged  $75 \pm 7$  (SD), with all cows first inseminated between 61 and 107 days (90% were inseminated between 61 and 82 DIM). Pregnancy rates at days 33 and 61 averaged in the low to mid 30% range, with pregnancy loss averaging 10.2%. Cows having prior luteal activity before treatment ( $65 \pm 7$  DIM), as assessed by concentrations of progesterone in 3 blood samples collected during 28 days before treatment, averaged 83.7%. Regression of the CL in response to the PGF<sub>2 $\alpha$</sub>  of the Ovsynch protocol averaged 88.5% across locations.

### ***Detection of Anovulatory Cows***

Our first objective was to determine the accuracy of detecting anovulatory cows by means of heatmount detectors or a single ultrasound examination of ovarian structures in comparison with blood serum concentrations of progesterone. Both assessments were made at all 6 locations. Overall, relative to 3 blood serum concentrations assessed during 28 days before treatment, heatmount detectors and a single ultrasound exam of ovarian structures underestimated both anovulation and previous luteal or cycling activity. Relative to serum progesterone patterns, however, overall accuracy of heatmount detectors was 71.4% and that of a single ultrasound examination was 84%. Ultrasound differed ( $P < 0.001$ ) from heatmount detectors in every category except for apparent prevalence, including being more ( $P < 0.001$ ) sensitive and specific than heatmount detectors, with the Kappa coefficient (0.52) in the good-agreement range compared with detectors (0.11). Heatmount detectors had greater ( $P < 0.001$ ) rates of false positives (identifying incorrectly prior estrual activity and subsequent luteal activity) and false negatives (missing prior estrual activity and subsequent luteal activity).

### ***Ovarian Characteristics in Response to Progesterone***

Our second objective was to determine the effects of progesterone (CIDR insert) in cows identified with or without a CL at the onset of the Ovsynch protocol. This assessment was made by a single ultrasound examination of ovaries at all 6 locations, and as cited earlier, some cows were not correctly categorized by the single examination. Incidence of ovulation in response to the first GnRH injection was less ( $P < 0.05$ ) in cows having no CL and treated with progesterone (CIDR insert) than in no CL cows not receiving a CIDR (Table 2). Average incidence of ovulation was less ( $P < 0.001$ ) for cows having a CL than for those without a CL (Table 2). Concentrations of progesterone for cows in the 3 previous classifications at onset of treatment were  $0.7 \pm 0.2$ ,  $0.6 \pm 0.2$ , and  $4.0 \pm 0.1$  ng/mL, respectively.

Diameter of the ovulatory follicle, assessed before the second GnRH injection, was smaller ( $P < 0.001$ ) in cows with a CL present than for those with no CL, regardless of CIDR treatment (Table 2). Incidence of ovulation of that follicle, assessed 5 days later (after TAI), did not differ among cows having or not having a CL (Table 2) at the onset of treatment.

### ***Fertility in Response to Progesterone***

Pregnancy rates at days 33 and 61 and pregnancy loss in 1,068 cows (4 were culled before pregnancy diagnosis) during that interval are summarized in Table 2 for all 6 locations. Cows having a CL present at initiation of treatment had greater ( $P < 0.05$ ) pregnancy rates at days 33 and 61 than those not having a CL. However, pregnancy rate at day 33 in CL absent cows treated with a CIDR did not differ from that in cows having a CL before treatment but was greater than for CL absent cows not treated with a CIDR. Cows requiring no calving assistance (calving difficulty score [CDS] = 1) subsequently had greater

( $P < 0.05$ ) pregnancy rates than those requiring some (CDS > 1) assistance (42.7%;  $n = 693$  vs. 34.3%;  $n = 375$ ), respectively.

Pregnancy rates at day 61 followed the same pattern as those at day 33 (Table 2). Overall pregnancy loss was 10.4% and tended ( $P < 0.10$ ) to be less in cows having a CL at the onset of treatment compared with cows not having a CL (Table 2).

### ***Time of Insemination***

Our third objective was to determine whether time of AI relative to the second GnRH injection (0 vs. 24 hours) altered pregnancy rates (Table 3). The largest difference (5.4 percentage points in 24 hours) in pregnancy rate at day 33 for timing of AI was observed in CL present cows. Otherwise, time of AI had no little effect on pregnancy outcome measured at day 33. Cows having a CL present at treatment initiation had greater ( $P < 0.05$ ) pregnancy rates than CL absent cows not treated with a CIDR when inseminated at 24 hours, but not 0 hours after the second GnRH injection.

A CIDR treatment  $\times$  location interaction tended ( $P = 0.085$ ) to occur (Table 3) for pregnancy rates at day 33. Pregnancy rates for cows treated with a CIDR insert were greater at 4 locations than non-CIDR treated cows, but were less than non-CIDR treated cows at the other 2 locations. An interaction of location  $\times$  time of AI tended ( $P = 0.12$ ) to occur, in which pregnancy rates for cows inseminated at 0 hour were numerically greater at 3 locations, but lesser at 3 other locations. Overall, pregnancy rates at 24 hours were numerically greater than those made at 0 hour (37.4%,  $n = 530$  vs. 34%,  $n = 538$ ), respectively.

### ***Concentrations of Progesterone***

The relationship of pretreatment cycling status and presence of high vs. low concentra-

tions of progesterone before the before injection of PGF<sub>2 $\alpha$</sub>  injection of Ovsynch is examined further in Table 4. Because of the inaccuracy of identifying cycling status by ultrasound relative to blood concentrations of progesterone in these cows, 55.5% of 155 CL absent cows were truly not cycling and treated with a CIDR. Further, 44% of 116 CL absent cows were truly not cycling and assigned to the no CIDR treatment. These 2 groups of cows are designated by their pretreatment cycling status in Table 4

Effects of treatment of anovulatory cows based on serum concentrations of progesterone are summarized in the top 3 lines of Table 4, and treatment effects on previously cycling cows are summarized in the bottom 3 lines of Table 4. When only previously anovulatory cows were treated or not treated with a CIDR, their pregnancy rates did not differ from those in CL present cows regardless of progesterone concentrations at the time of the PGF<sub>2 $\alpha$</sub>  injection (Table 4). In contrast, regardless of progesterone concentrations at the time of the PGF<sub>2 $\alpha$</sub>  injection, CL absent cows treated with a CIDR and CL cows had greater pregnancy rates than the CL absent cows not treated with a CIDR (Table 4). Overall, cows having prior cycling activity, assessed by blood progesterone before treatment, had 53% greater ( $P < 0.001$ ) pregnancy rates at day 33 than those without prior luteal activity (37.8 vs. 24.7%; Table 4).

Treatment with the CIDR insert in a previous study improved pregnancy rates in cows that were previously noncycling without a functional CL before the PGF<sub>2 $\alpha$</sub>  injection, but not in the present study (Table 4). Further, CIDR treatment in previously cycling cows having low concentrations of progesterone before PGF<sub>2 $\alpha$</sub>  injection (early CL regression) had numerically greater pregnancy rates in the present study as in a previous study. Interpretation of the results in the previous study indi-

cated that the CIDR insert would improve conception rates in cows having no active CL or low progesterone before  $\text{PGF}_{2\alpha}$  injection regardless of previously cycling or luteal status, whereas in the present study, we could only verify improved fertility for previously cycling cows having low progesterone (early CL regression). Although interpretation of results in the present study relative to luteal status before  $\text{PGF}_{2\alpha}$  injection should be conservative because luteal status was confounded with CIDR-supplemented progesterone concentrations, nearly 26% of the CL absent cows treated with a CIDR had concentrations of progesterone  $< 1$  ng/mL when the insert was removed. Clearly, these cows had no CL at this time. Concentrations of progesterone in cows bearing a luteal structure are generally not different before and after the insert is removed.

Concentrations of progesterone in blood serum 2 days before the second GnRH injection (day of  $\text{PGF}_{2\alpha}$  injection of Ovsynch and removal of CIDR inserts), before the second GnRH injection, and 5 and 12 days later are illustrated in Figure 2. At insert removal, cows treated with CIDR inserts had concentrations of progesterone that did not differ from those in cows without inserts, but both treatments had less ( $P < 0.05$ ) progesterone than in CL present cows. This same pattern existed 48 hours later when the second GnRH injection was given (0 h). At 5 days after AI, no differences in progesterone concentration were detected among treatments, but at 12 days after the second GnRH injection or 11 to 12 days after AI, CL absent cows, regardless of CIDR treatment, had greater ( $P < 0.05$ ) concentrations of progesterone than CL present cows. The greater progesterone 12 days

later is consistent with an earlier report in which cows treated with CIDR inserts during the Ovsynch protocol had greater progesterone concentrations than controls. It seems that maturing follicles exposed to progesterone during CIDR treatment may, after ovulation, differentiate into CL having greater progesterone secretory capacity.

As expected, pregnancy status had no effect on concentrations of progesterone at 5 days after the second GnRH injection, but at 12 days, pregnant cows had greater ( $P < 0.001$ ) concentrations of progesterone than nonpregnant cows ( $4.7 \pm 0.2$ ;  $n = 686$  vs.  $3.6 \pm 0.1$  ng/mL;  $n = 381$ ).

In summary, identifying cows with a CL was more accurate after the single ultrasound examination than during the 28 days when heatmount detectors were applied to cows. When lactating dairy cows were found to have no CL (but had evidence of increased concentrations of progesterone in blood serum during 28 days before initiating the Ovsynch protocol) at the onset of the Ovsynch protocol and treated with progesterone (via a CIDR), pregnancy rates were greater than similar CL absent cows not treated with a CIDR. Pregnancy rates of the former did not differ from cows having a CL at the initiation of the TAI protocol. Our study finds no evidence that including progesterone in a TAI protocol for previously noncycling cows is warranted, regardless of luteal status before the  $\text{PGF}_{2\alpha}$  injection of the Ovsynch protocol. Insemination of cows at 0 vs. 24 hours after the second GnRH injection did not differ significantly, even though a numerical advantage (5.4 percentage points) occurred for CL present cows inseminated at 24 vs. 0 hours.

**Table 1. Selected Outcomes of Lactating Dairy Cows Enrolled at Each Location**

Item	Location						Total
	IN	KS	MI	MN	MO	WI	
Cows enrolled, no.	80	217	153	194	242	186	1,072
Calving difficulty score <sup>1</sup>	1.5 <sup>a</sup>	1.3 <sup>a,b</sup>	1.2 <sup>c</sup>	1.7 <sup>d</sup>	1.4 <sup>a</sup>	1.3 <sup>b,c</sup>	1.3 <sup>**</sup>
Body condition score post-calving <sup>1</sup>	2.9 <sup>a</sup>	2.6 <sup>b</sup>	2.7 <sup>c</sup>	3.1 <sup>d</sup>	2.9 <sup>a</sup>	...	2.8 <sup>**</sup>
Body condition score at Presynch <sup>1,2</sup>	...	2.3 <sup>a</sup>	2.4 <sup>b</sup>	2.8 <sup>c</sup>	2.7 <sup>d</sup>	2.9 <sup>c</sup>	2.6 <sup>**</sup>
Days postpartum at first AI <sup>3</sup>	72 <sup>a</sup>	79 <sup>b</sup>	82 <sup>c</sup>	69 <sup>d</sup>	75 <sup>c</sup>	72 <sup>a</sup>	75 <sup>**</sup>
Pregnancy rate at 33 d <sup>4</sup> , %	37.5	42.9	36.0	29.4	33.1	36.3	35.7
Pregnancy rate at 61 d <sup>4</sup> , %	37.5 <sup>a</sup>	37.3 <sup>a</sup>	33.3 <sup>a</sup>	22.7 <sup>b</sup>	29.8 <sup>a,b</sup>	35.2 <sup>a</sup>	32.0 <sup>*</sup>
Pregnancy loss (33 to 61 d), %	0.0 <sup>a,c</sup>	12.9 <sup>b</sup>	7.3 <sup>a,c</sup>	22.8 <sup>b</sup>	10.0 <sup>c</sup>	3.0 <sup>c</sup>	10.2 <sup>*</sup>
Luteal activity before treatment <sup>5</sup> , %	92.5 <sup>a</sup>	91.2 <sup>a</sup>	89.5 <sup>a</sup>	69.1 <sup>b</sup>	79.3 <sup>c</sup>	87.3 <sup>a,c</sup>	83.7 <sup>**</sup>
Regression of CL <sup>6</sup> , %	88.4 <sup>a,b</sup>	86.7 <sup>b,c</sup>	87.4 <sup>b</sup>	81.9 <sup>b</sup>	94.8 <sup>a</sup>	90.6 <sup>a,c</sup>	88.5 <sup>**</sup>

<sup>\*\*</sup>Location effect ( $P < 0.01$ ).

<sup>\*</sup>Location effect ( $P < 0.05$ ).

<sup>a,b,c,d,e</sup>Location values having different superscript letters differ ( $P \leq 0.05$ ).

<sup>1</sup>Range of 1 to 5. Standard deviations ranged from 0.44 to 0.48.

<sup>2</sup>Assessed before first Presynch PGF<sub>2α</sub> injection.

<sup>3</sup>Standard deviation was 7.0.

<sup>4</sup>Determined by transrectal ultrasonography after the first postpartum AI.

<sup>5</sup>Based on progesterone concentrations measured in a total of 3 blood samples collected before each Presynch PGF<sub>2α</sub> injection and before ultrasonography at the time of the first GnRH injection of the Ovsynch protocol. Cutoff values for luteal activity were based on progesterone  $\geq 1$  ng/mL and  $< 1$  ng/mL for no luteal activity.

<sup>6</sup>Cows having elevated concentrations of progesterone 48 to 72 hours before AI in which blood progesterone was  $< 1$  ng/mL at 48 hours after the PGF<sub>2α</sub> injection of the Ovsynch protocol.



**Table 2. Ovarian Characteristics and Fertility in Response to Presence or Absence of a Corpus Luteum (CL) Assessed by Transrectal Ultrasonography and Progesterone Treatment (CIDR insert) at the Onset of the Ovsynch Protocol**

Item	CL absent <sup>1</sup>		CL present <sup>1</sup>
	No CIDR	CIDR	
Ovulation after first GnRH <sup>2</sup> , % (no.)	76.8 <sup>a</sup> (69)	47.1 <sup>b</sup> (104)	43.4 <sup>c,x</sup> (604)
Presence of CL before PGF <sub>2α</sub> <sup>2</sup> , % (no.)	76.8 <sup>a</sup> (69)	51.0 <sup>b</sup> (104)	95.7 <sup>c,x</sup> (610)
Corpus luteum before PGF <sub>2α</sub> <sup>3</sup>	0.9 <sup>a</sup> ± 0.1 (55) <sup>4</sup>	0.7 <sup>a</sup> ± 0.1(78)	1.4 <sup>b</sup> ± 0.1 (450)
Diameter of ovulatory follicle before second GnRH <sup>2</sup> , mm	16.7 <sup>a</sup> ± 0.4 (65) <sup>4</sup>	16.2 <sup>a</sup> ± 0.4 (103)	15.2 <sup>b,x</sup> ± 0.1 (588)
Ovulation after second GnRH <sup>2</sup> , % (no.)	77.9 (68)	75.0 (104)	80.7 (605)
Pregnancy rate at day 33, % (no.)	24.1 <sup>a</sup> (116)	32.3 <sup>b</sup> (155)	38.0 <sup>b,x</sup> (797)
Pregnancy rate at day 61, % (no.)	20.7 <sup>a</sup> (116)	29.0 <sup>b</sup> (155)	34.3 <sup>b,x</sup> (797)
Pregnancy loss from day 33 to 61, % (no.)	14.3 (28)	10.0 (50)	9.9 <sup>y</sup> (303)

<sup>a,b,c</sup>Means having different superscript letters differ ( $P \leq 0.05$ ).

<sup>x</sup>Different ( $P \leq 0.05$ ) from CL absent cows.

<sup>y</sup>Tended ( $P \leq 0.10$ ) to differ from CL absent cows.

<sup>1</sup>Absence or presence of a CL assessed by transrectal ultrasonography at the first GnRH injection of the Ovsynch protocol.

<sup>2</sup>Assessed at only 3 of 6 locations.

<sup>3</sup>Assessed at only 2 of 6 locations.

<sup>4</sup>Mean ± SE and number of observations.

**Table 3. Pregnancy Rates at Day 33 After Timed Insemination on the Basis of Time of AI After the Second GnRH Injection and Location**

Item	CL absent <sup>1</sup>		CL present <sup>1</sup>	Overall
	No CIDR	CIDR		
AI time, hour after second GnRH	----- % (no.) -----			
0	24.1 <sup>a</sup> (54)	33.8 <sup>a</sup> (74)	35.4 <sup>a</sup> (401)	34.0 (530)
24	24.2 <sup>a</sup> (62)	30.9 <sup>a</sup> (81)	40.8 <sup>b,x</sup> (395)	37.4 (538)
Location <sup>2</sup>				
IN	22.2 (9)	18.8 (16)	45.5 (55)	37.5 (80)
KS	21.7 (23)	26.1 (23)	48.0 (171)	42.9 (217)
MI	12.5 (16)	58.3 (12)	36.8 (125)	35.9 (153)
MN	21.6 (37)	32.4 (34)	30.9 (123)	29.4 (194)
MO	35.0 (20)	29.0 (62)	34.4 (160)	33.1 (242)
WI	36.4 (11)	62.5 (8)	35.2 (162)	36.3 (182)

<sup>a,b</sup>Means within AI timing having different superscript letters differ ( $P \leq 0.05$ ).

<sup>x</sup>Different ( $P \leq 0.05$ ) from corpus luteum (CL) absent cows at 24 hours.

<sup>1</sup>Absence or presence of a CL assessed by transrectal ultrasonography at the first GnRH injection of the Ovsynch protocol.

<sup>2</sup>Interaction ( $P = 0.08$ ) of treatment  $\times$  location.

**Table 4. Pregnancy Rates at Day 33 After Timed Insemination According to Luteal Activity Before Treatment and Concentrations of Progesterone at the Time of PGF<sub>2 $\alpha$</sub>  Injection**

Pretreatment cycling status <sup>1</sup>	Serum progesterone before PGF <sub>2<math>\alpha</math></sub> <sup>2</sup>	CL absent <sup>3</sup>			Pretreatment cycling total
		No CIDR	CIDR	CL present <sup>3</sup>	
		----- % (no.) -----			
Noncycling	High	29.4 (34)	32.1 (56)	32.0 <sup>a,b</sup> (25)	
	Low	17.6 (17)	6.7 (30)	16.7 <sup>a,x</sup> (12)	
	Total	25.4 <sup>a</sup> (51)	23.3 <sup>a</sup> (86)	27.0 <sup>a</sup> (37)	24.7 (174)
Cycling	High	25.9 (54)	44.1 (59)	40.2 <sup>b</sup> (651)	
	Low	9.1 (11)	40.0 (10)	28.7 <sup>a,b</sup> (108)	
	Total	23.1 <sup>a</sup> (65)	43.5 <sup>b</sup> (69)	42.8 <sup>b</sup> (759)	37.8* (893)

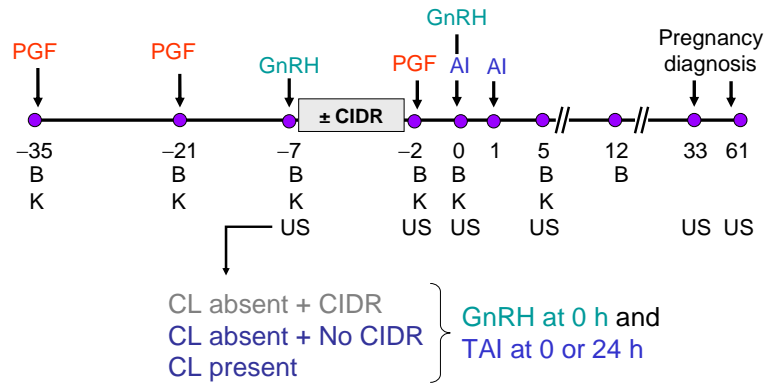
<sup>a,b</sup>Treatment  $\times$  cycling status interaction ( $P = 0.08$ ).

\*Differed ( $P < 0.05$ ) from noncycling cows.

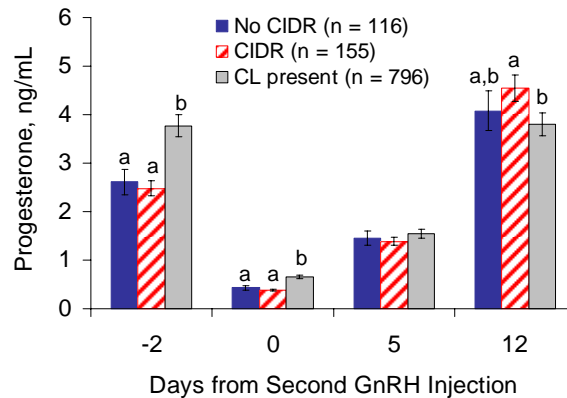
<sup>1</sup>Based on progesterone concentrations measured in 3 blood serum samples collected before each Presynch PGF<sub>2 $\alpha$</sub>  injection and before the first GnRH injection of the Ovsynch protocol.

<sup>2</sup>Low = concentrations of progesterone  $< 1$  ng/mL, and high =  $\geq 1$  ng/mL.

<sup>3</sup>Absence or presence of a corpus luteum (CL) assessed by transrectal ultrasonography at the first GnRH injection of the Ovsynch protocol. Some CIDR-treated cows may have had elevated progesterone because of the CIDR insert.



**Figure 1. Experimental Protocol Showing Design of Treatments.** Heatmount detectors (K) were applied to detect mounted activity. Activated heatmount detectors were replaced at each evaluation. Blood (B) was collected before various injections and twice post AI. Presence of a corpus luteum (CL) was detected by transrectal ultrasonography (US) and, alternatively, cows without a CL received a progesterone-releasing intravaginal controlled internal drug release (CIDR) insert. Cows having a CL present received no CIDR. All cows received GnRH at 48 hours after PGF<sub>2α</sub> (PGF) and were inseminated before the second GnRH injection (0 hour) or 24 hours later in the 3 treatments. Pregnancy was diagnosed at 33 days after timed AI and reconfirmed at 61 days after timed AI.



**Figure 2. Concentrations of Progesterone in Serum of Lactating Dairy Cows Before Injections of PGF<sub>2α</sub> [time of progesterone (CIDR) Insert Removal; day -2] and the Second GnRH Injection (day 0), and at 5 and 12 Days After GnRH.** <sup>a,b</sup> Means having different superscript letters differ ( $P < 0.05$ ).

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

*B. S. Buttrey, M. G. Burns, and J. S. Stevenson*

### **Summary**

Experiments have shown human chorionic gonadotropin (hCG) to be more effective than gonadotropin 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<sub>2α</sub> (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<sub>2α</sub> 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  $\times$  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  $\times$  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  $2.2 \pm 1\%$ . 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  $\times$  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  $\times$  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  $\geq 2$  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  $\times$  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  $\times$  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 <sup>1</sup>	Postpartum insemination number			Total
	1	2	≥ 3	
	----- % (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 <sup>a</sup> (413)
Control	94.6 (147)	98.4 (63)	94.8 (193)	95.3 (403)
Total	92.1 (457)	95.2 (210)	94.9 (569)	

<sup>a</sup>Tended ( $P = 0.06$ ) to differ from control.

<sup>1</sup>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 <sup>1</sup>	Postpartum insemination number				Total
	2	3	4	≥ 5	
	----- % (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 <sup>a</sup> (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)	

<sup>a</sup>Tended ( $P = 0.08$ ) to differ from saline.

<sup>1</sup>Cows were treated once with hCG, GnRH, or served as untreated controls 7 days before pregnancy diagnosis.



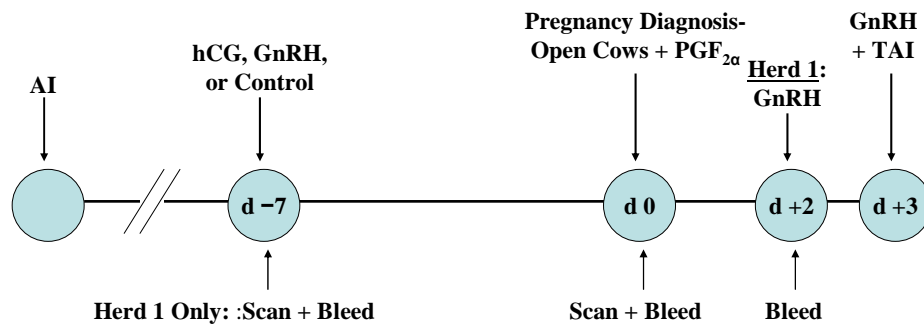
**Table 3. Blood Serum Concentrations of Progesterone 7 Days After Treatment Based on Pregnancy Status and Number of Corpora Lutea (CL) at Time of Treatment (Exp. 3)**

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

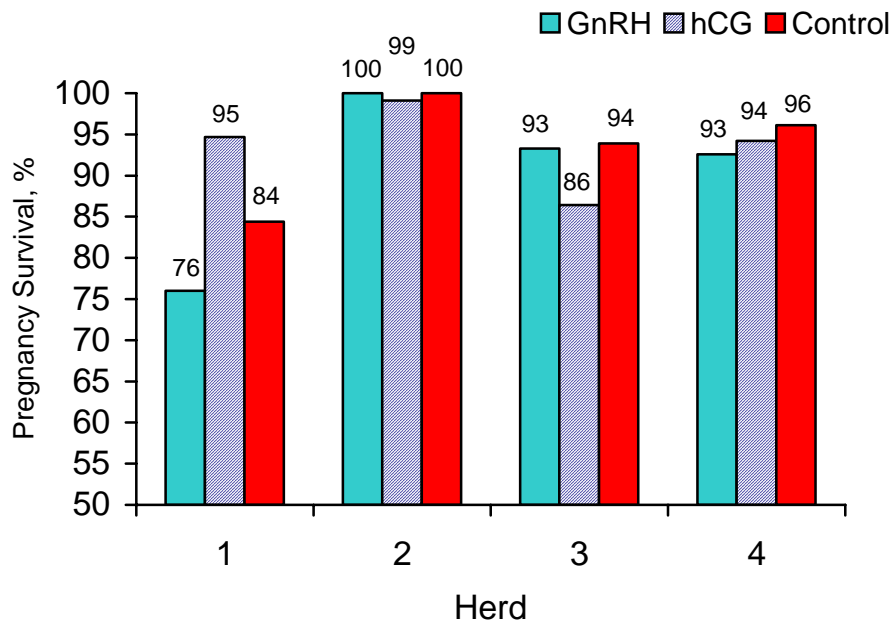
<sup>a,b,c</sup> Means having different superscript letters within item differ ( $P < 0.001$ ).

<sup>1</sup>Adjusted for concentrations of progesterone at the time of treatment.

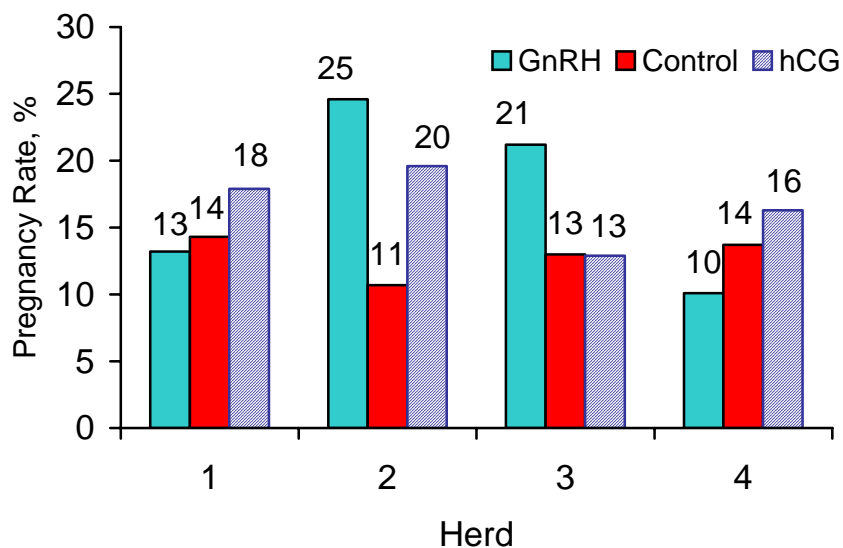
<sup>2</sup>Cows were treated once with hCG, GnRH, or served as untreated controls 7 days before pregnancy diagnosis.



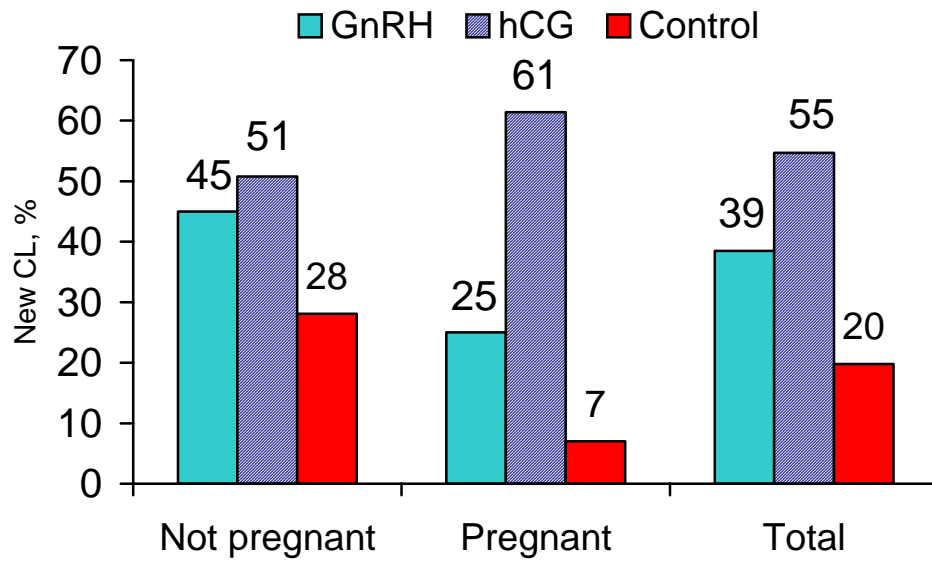
**Figure 1. Experimental Design.** AI = artificial insemination; TAI = timed AI; GnRH = gonadotropin releasing hormone; PGF<sub>2α</sub> = prostaglandin F<sub>2α</sub>; and hCG = human chorionic gonadotropin.



**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  $\times$  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  $\times$  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  $\times$  pregnancy status interaction was detected.

*Dairy Research 2007*

**TIMED ARTIFICIAL INSEMINATION CONCEPTION RATES IN RESPONSE TO A PROGESTERONE INSERT IN LACTATING DAIRY COWS**

*C. A. Martel, B. S. Buttrey, M. G. Burns, W. E. Brown, and J. S. Stevenson*

**Summary**

Our objective was to determine the effectiveness of exogenous progesterone in the form of an intravaginal insert (controlled internal drug release, CIDR) in conjunction with an ovulation-synchronization protocol in lactating dairy cows. Cows received a Presynch protocol (two injections of prostaglandin F<sub>2α</sub> [PGF<sub>2α</sub>] 14 days apart) beginning 30 and 36 days in milk, respectively, in two herds. Cows were inseminated after the second Presynch injection when estrus was detected. Remaining cows were treated with the Ovsynch protocol, and alternate cows were assigned randomly to receive a progesterone insert (CIDR). Blood was collected, and body condition scores (BCS) were assigned to treated cows. Pregnancy status was confirmed by palpation on day 38 post timed AI (TAI) and verified again 4 weeks later. Progesterone increased conception rates in treated cows when compared with controls (38 vs. 24%), but did not differ from early inseminated cows (38%). Pregnancy loss was numerically less in progesterone-treated cows than in controls (4.4 vs. 11.8%).

(Key words: CIDR, Ovsynch, GnRH, PGF<sub>2α</sub>.)

**Introduction**

Fate of a dairy cow lies in her ability to reproduce; however, in an constantly changing dairy industry, reproductive performance has dramatically declined. As a means to manage reproductive programs, ovulation-synchroni-

zation protocols were developed. Development of the Ovsynch protocol opened new doors for the dairy producer. Variations of the Ovsynch protocol have been tested to synchronize ovulation by altering timing of gonadotropin releasing hormone (GnRH) and PGF<sub>2α</sub> injections. Because GnRH is used to control follicular development and induce ovulation of a dominant follicle and PGF<sub>2α</sub> causes regression of the corpus luteum (CL), timing of injections can further improve fertility when used at certain stages of the estrous cycle. Follicular development and early maintenance of pregnancy requires endogenous progesterone to be secreted by the CL. Progesterone prevents the return of estrus and is used to synchronize estrus. Integration of exogenous progesterone into a synchronization protocol can prevent estrus in cows before insert removal and TAI

Other studies have found progesterone treatment of cows before first service to improve TAI conception rates, regardless of whether they had normal estrous cycles before AI. In a previous study, cows were inseminated during 28 days while the Presynch protocol was applied to them (two injections of PGF<sub>2α</sub> administered 14 days apart). Those not yet inseminated were then treated with a progesterone insert as part of the Ovsynch protocol. Resulting TAI conception rates were improved compared with non-progesterone treated cows. Our objective was to apply progesterone to cows (as part of the Ovsynch protocol) that had not been inseminated during 12 days after the second of two Presynch injec-

tions and determine subsequent TAI conception rates.

### Procedures

Lactating dairy cows on 2 dairy farms were enrolled in a Presynch + Ovsynch protocol after parturition and assigned randomly (based on lactation number: 1, 2, or 3+) to receive either of two treatments: CIDR or no CIDR if they failed to express estrus and were not inseminated after the second of two Presynch injections (Figure 1). Cows received 2 initial Presynch injections of PGF<sub>2α</sub>. Cows detected in estrus after the second Presynch injection of PGF<sub>2α</sub> were inseminated. Remaining cows received the standard Ovsynch protocol and were injected with GnRH and either received a progesterone insert (CIDR) for 7 days or served as controls. After CIDR removal, all cows received an injection of PGF<sub>2α</sub>. Cows were inseminated at 72 hours after PGF<sub>2α</sub> and given a second injection of GnRH. Blood samples were collected to analyze concentrations of progesterone before the second Presynch injection in all cows. No further blood collection occurred for cows inseminated during the 12 days between the second Presynch injection and initiation of treatment. Cows assigned to treatment were blood-sampled before the CIDR insert was placed and again 11 days after TAI to determine cycling status of each cow and effect of treatment on post-AI concentrations of progesterone. Body condition scores (1 = thin and 5 = fat) were assigned at the onset of treatment. Cows were diagnosed pregnant by palpation beginning 38 days after AI.

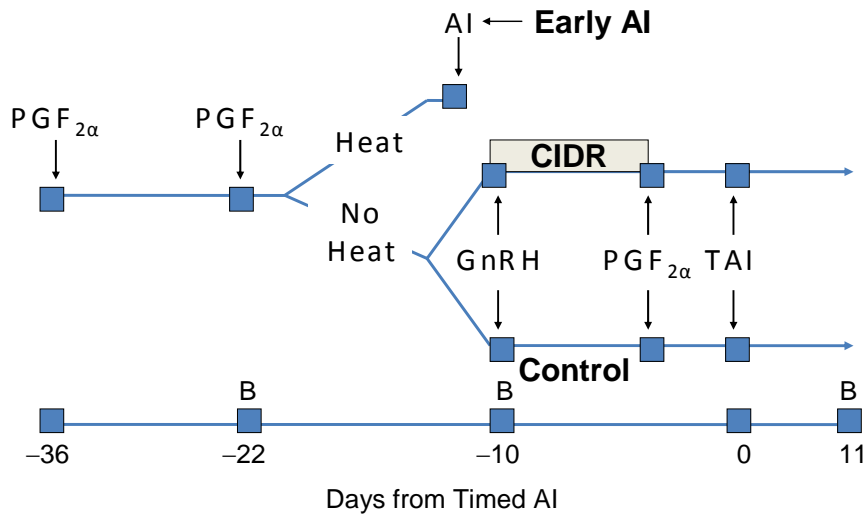
### Results and Discussion

Serum progesterone concentrations revealed that the majority of cows were cycling

before TAI. At location 1, 39.6% of the cows were inseminated early between the second Presynch PGF<sub>2α</sub> injection and the onset of the Ovsynch protocol, whereas 26.5% were inseminated early at location 2. Of the remaining 333 cows, 231 (69.4%) were found to have elevated progesterone in either or both samples collected before treatment, indicating that about 30% of the treated cows were not cycling or anovulatory before treatment.

Overall conception rates did not differ between herds (32 vs. 34%) or between cows that were cycling and not cycling (34 vs. 28%; Table 1) before treatment. Younger cows had greater ( $P < 0.05$ ) conception rates than older cows (Table 1). Cows with more body condition had greater conception rates. Cows having a BCS  $< 1.75$  averaged 12.5 percentage points less in conception rate than those cows having BCS  $\geq 2.25$  (Table 1).

Timed AI conception rates were greater in cows treated with the progesterone insert compared with controls (38 vs. 25%), regardless of cycling status (Table 2). Early inseminated cows had conception rates similar to CIDR-treated cows. Pregnancy loss in CIDR-treated cows was similar to early bred cows, but less than controls. Concentrations of progesterone 11 days after timed AI tended to be greater than those in controls. Our study shows that increased conception rates can be achieved by using a progesterone insert in a reduced population of cows not yet inseminated. Further, thinner cows had poorer TAI conception rates, and cows in their first lactation were more fertile than older cows. This study is part of a larger, multi-state study in which a similar protocol was applied to cows in Arizona, California, and Wisconsin. Results of the entire study are forthcoming.



**Figure 1. Experimental Design of Treatments.** PGF<sub>2α</sub> = prostaglandin F<sub>2α</sub>; CIDR = progesterone releasing intravaginal insert; GnRH = gonadotropin releasing hormone; AI = artificial insemination; and TAI = timed AI.

**Table 1. Location, Cycling Status, Lactation Number, and Body Condition Effects on AI Conception Rates**

Item	No. of cows	AI conception rate, %
Location		
1	197	32.0
2	291	33.7
Cycling status <sup>1</sup>		
Yes	386	34.4
No	102	27.5
Lactation		
1	203	37.4 <sup>a</sup>
2+	285	29.8
Body condition score <sup>2</sup>		
< 1.75	166	26.5
= 2.00	140	32.9
≥ 2.25	182	39.0

<sup>a</sup>Different ( $P < 0.05$ ) from 2+ lactation cows.

<sup>1</sup>Based on serum concentrations of progesterone in blood samples collected before the second Presynch PGF<sub>2α</sub> injection and before the onset of the Ovsynch protocol in progesterone insert and control cows only.

<sup>2</sup>Assessed before the second Presynch PGF<sub>2α</sub> injection.

**Table 2. Conception Rates, Pregnancy Loss, and Serum Progesterone After AI in Lactating Dairy Cows in Response to Treatment**

Treatment <sup>1</sup>	Cycling status	AI conception rate	Pregnancy loss	Serum progesterone, ng/mL
		----- (No./no.) % -----		Mean ± SE (no.)
Early AI <sup>2</sup>	Yes	(56/155) 36.1 <sup>a</sup>	(4/55) 7.3	...
Progesterone insert	Yes	(45/122) 36.9	(2/45) 4.4	4.8 ± 0.3 (123)
	No	(21/52) 40.4	(2/21) 9.5	4.8 ± 0.4 (52)
	Total	(66/174) 37.9 <sup>a</sup>	(4/66) 6.1	4.8 <sup>x</sup> ± 0.2 (175)
Control	Yes	(32/109) 29.4	(4/32) 12.5	4.4 ± 0.2 (108)
	No	(7/50) 14.0	(1/7) 14.3	4.2 ± 0.4 (51)
	Total	(39/159) 24.5 <sup>b</sup>	(5/39) 12.8	4.3 <sup>y</sup> ± 0.2 (159)

<sup>a,b</sup>Mean percentages having different superscript letters differ ( $P < 0.01$ ).

<sup>x,y</sup>Mean percentages having different superscript letters tended ( $P < 0.10$ ) to differ.

<sup>1</sup>Cows in the Early AI treatment were inseminated after detected estrus following the second Presynch PGF<sub>2α</sub> injection. The Ovsynch protocol was applied to the remaining cows not inseminated, of which approximately one-half were treated either with a progesterone insert (CIDR) for 7 days beginning with the first GnRH injection or served as controls.

<sup>2</sup>At location 1, 37.1% of the cows were inseminated early between the second Presynch PGF<sub>2α</sub> injection and the onset of the Ovsynch protocol, whereas 24.5% were inseminated early at location 2.

*Dairy Day 2007*

## **IMPACT OF SLOPE AND PIPE DIAMETER ON FLUSH PLUME DESIGN**

*J. P. Harner, J. F. Smith, and M. J. Brouk*

### **Summary**

Manning's equation provides a method to evaluate the flow characteristics of a flush plume system used to move a diluted, sand-laden manure stream from a freestall building to sand or solid separation equipment. Evaluation of a 16, 18, and 24-inch plume showed pipe slope is critical in maintaining a 5 feet per second water velocity through the pipe. A 24 inch or larger plume placed on a 0.5% slope is able to obtain water velocity of 5 feet per second if the pump capacity exceeds 3,600 gpm. The flow velocity never reached or exceeded 5 feet per second in a 16- or 18-inch pipe placed on a 0.5% slope, regardless of the pump capacity. A 16-, 18- or 24-inch pipe laid on a 1% slope could obtain a water velocity of 5 feet per second if the pump capacity exceeded 1,500 gallons/minute.

(Key Words: sand, plume, flush, sand separation.)

### **Introduction**

Sand is the preferred bedding option on many dairies in spite of the challenges with sand laden manure. The abrasiveness of the sand causes increase wear on equipment and is difficult to remove from containment structures. Many dairies are using passive (gravity) or non-passive (mechanical) sand separation systems to reduce the volume of sand entering further storage structures. Both separation systems required diluting the sand-laden manure with fresh or recycled water. A water to sand

laden manure dilution ratio of 5:1 is representative of flush streams (Wedel, A.W. and W.G. Bickert. 1996. Separating sand from sand-laden manure: factors affecting the process. Paper No. 1996-4016. ASABE, St Joseph, MI.). This equates to a water:sand ratio of 20:50 depending on the sand usage in the stall and water volume per square feet of alley space being flushed. Different types of settling processes have been used to recover sand from a flush stream.

Often, existing dairies are unable to retrofit their facilities to a flush system that would enable them to recover sand. A flush plume system provides an alternative on these dairies. In this system, manure is scraped into a plume, and water from a containment structure is used to erode or move the sand-laden manure to a sand separation system. A pump is used to recycle water from a containment structure to the plume. The plume openings allow manure to drop inside a pipe but prevent the system from being pressurized. Water and sand-laden manure move through a plume via gravity to the separation or storage area. Pipe slope, diameter, and surface roughness influences water velocity and volume in open channel flow. Optimizing the plume system on existing dairies may not be practical because topography and existing building evaluations may limit pipe slope. Pipe diameter and surface roughness, however, are controllable factors even in a retrofit situation. The objective of this study was to evaluate the effect of pipe diameters and pipe slope on water velocity and volume.



## Procedures

The common design equation for fluid flow in open channels and pipes is known as Manning's equation. The equation considers piping material, pipe slope, wetted perimeter, and pipe cross-sectional area. This equation is used for open channel flow in which the flow rate is controlled by gravity as compared with a pump system where head pressure forces liquid through an enclosed conveying channel.

Manning's equation is represented by:

$$Q = (1.486 \times S^{0.5} \times A^{1.667}) / (n \times P^{0.667}),$$

where

Q = pipe flow (cubic feet per second),

S = slope (ft/ft),

A = water cross sectional area (sq ft),

n = pipe surface coefficient, and

P = wetted perimeter (ft).

Normally, this equation is used for open channel or full pipe flow; however, in plume design only partial flow is considered. Geometrical equations can be used to estimate the cross-sectional area of a pipe with partial flow and the wetted perimeter based on the water depth in the pipe. The surface coefficient is a function of the type of pipe. Smooth plastic pipe has a coefficient ranging from 0.009 to 0.015. For this study, a coefficient of 0.013 was used based on earlier work (Metcalf & Eddy, Inc. 1981. *Wastewater Engineering: Treatment Disposal and Reuse*. New York, McGraw-Hill). Pipe slopes of 0.5 or 1% were evaluated and reflected normal construction practices. Pipe diameters selected were 24, 18, and 16 inches. In order to compare different pipes, percentage depth of water flow in the pipe was used. Water flow depths of 25, 50, 75, and 100% of pipe diameter were considered. At 100% of pipe diameter, the pipe would be flowing at full capacity.

## Results

Table 1 summarizes the flow velocity (feet/second) and flow rate (gallons/minute) as a function of pipe diameter, pipe slope, and depth of flow. Recommended pipe flows are 5 to 8 feet/second in plume design with handling sand-laden manure (Wedel, A.W. 2000. *Hydraulic conveyance of sand-laden dairy manure in collection channels*. Paper No. 00-4106. ASABE, St Joseph. MI). Further, "a flow velocity of 5 feet/second will initiate scour." At 5 feet/second, 4 mesh grains will not be completely suspended in water but will bounce along the bottom of the plume. This flow velocity is obtainable when 18- or 16-inch pipes are placed on a 1% slope or a 24-inch pipe is used on either a 0.5 or 1% slope. Optimum flow velocity occurs when the water flow depth is 75% of the pipe diameter. The flow velocity at 100 and 50% flow depth is equal because the ratio of wetted perimeter to pipe cross sectional area is equal. Current recommendations indicate limiting pipe flow to 50% of the pipe diameter or half of the cross-sectional area. This should provide room in the pipe to temporarily store manure until the water erodes and moves the sand-laden manure to the separation system.

Table 1 also shows the flow volume necessary to sustain the flow velocity for the varying design parameters. Assuming a water flow depth of 50% and minimum water velocity of 5 feet/second, a 24-inch pipe on a 0.5% slope requires a minimum pump capacity of 3,600 gallons/minute. The pump capacity required for an 18- and 16-inch plume on a 1% slope is 2,400 and 1,700 gallons/minute, respectively. Further analysis indicates a minimum pump capacity for 16-, 18-, and 24-inch plumes is 1,500 gallons/minute if water velocity is maintained at 5 or greater feet/second. However, the water flow depth is less than 50% of the pipe diameter if the pipe has a 1%

slope. A plume 24 inches or larger is required if the pipe slope is 0.5%. The flow volume must equal or exceed 3,600 gallons/minute to maintain a velocity of 5 feet/second if the pipe slope is 0.5%. If a 1,500 gallons/minute pump is used with a 24 inch or larger plume on a 0.5% slope, then the water velocity will range between 4 and 5 feet/second. Some coarser sand may settle causing the pipe to plug if the water velocity is less than 5 feet/second.

Manning's equation provides a method to evaluate the flow characteristics of a flush plume system used to move a diluted sand-laden manure stream from a freestall building

to a sand or solid separation system. Evaluation of a 16-, 18-, and 24-inch plume showed that pipe slope is critical in maintaining a 5 feet/second water velocity through the pipe. This velocity could not be reached in a 16 or 18 inch plume if the pipe was placed on a 0.5% slope. A 24 inch or larger plume placed on a 0.5% slope is able to obtain 5 feet/second water velocity, but only if the pump capacity exceeds 3,600 gallons/minute. The design parameters indicate, if the site allows, that the least cost option is placing the plume on a 1% slope, because a pump with a 1,500 gallons/minute capacity is acceptable and the plume may be 16 to 24 inches wide.

**Table 1. Flow Velocity and Flow Volume Through Different Size Plume Pipes Placed on Either a 0.5 or 1% Slope**

Pipe slope (%)	Water flow depth (% of pipe diameter) (%)*	PVC pipe diameter					
		24 inches		18 inches		16 inches	
		Flow velocity (fps) <sup>1</sup>	Flow volume (gpm) <sup>2</sup>	Flow velocity (fps)	Flow volume (gpm)	Flow velocity (fps)	Flow volume (gpm)
0.5	100	5.1	7,200	4.2	3,400	3.9	2,400
	75	5.8	6,600	4.8	3,100	4.4	2,200
	50	5.1	3,600	4.2	1,700	3.9	1,200
	25	3.6	990	3.0	460	2.7	340
1	100	7.2	10,200	6.0	4,700	5.5	3,500
	75	8.2	9,300	6.8	4,300	6.2	3,200
	50	7.2	5,100	6.0	2,400	5.5	1,700
	25	5.1	1,400	4.2	650	3.9	470

\*This represents the percentage of the cross sectional area with water flow. For example, if the pipe is half full, then water flow depth is 50% of the pipe diameter. The water flow depth equals 100% of the pipe diameter if the pipe is flowing full.

<sup>1</sup>Feet per second.

<sup>2</sup>Gallons per minute.

*Dairy Day 2007*

## **IMPACT OF SEPARATOR EFFICIENCY AND REMOVED SOLIDS MOISTURE CONTENT ON MAKE-UP WATER REQUIREMENTS FOR SAND RECOVERY**

*J. P. Harner, J. F. Smith, and M. J. Brouk*

### **Summary**

Separator efficiency and moisture content of separated solids influence the make-up water requirements on a dairy seeking to utilize recycled water for sand reclamation. Additional water requirements range from 0 to 79 gallons/cow per day depending on the efficiency of the solid to liquid separation process. Make-up water includes any water stored in a containment structure, excluding urine. Final moisture content of the separated solids had marginal impact on the additional water requirements.

(Key Words: sand recovery, recycled water, separator efficiency.)

### **Introduction**

Cow comfort is often improved by bedding freestalls with sand. Many popular press articles, as well as scientific peer reviewed articles, have discussed the benefits of sand. Sand is not as readily available in all parts of North America, and cost varies from \$5 to \$25 per ton. Some producers are concerned about the agronomic impact of the sand-laden manure applied to fields. Other see an increase in maintenance cost of equipment when handling sand-laden manure because of sand abrasiveness. Another disadvantage is that sand tends to settle in undesirable areas, such as lagoons or digesters. These concerns highlight the importance of removing sand from the waste stream quickly and economically. Removing sand from the waste stream is accomplished

by diluting the sand-laden manure stream with water.

Both mechanical and passive (gravity) systems require addition of water. Fresh water is preferred; but generally, recycled water from a storage pond or lagoon is used. Use of recycled water reduces the total volume of liquid that must be applied to land. It should not reduce, however, the land base requirements for nutrient management plans. Excreted nutrients that must be applied to land are a function of excreted manure (number of cows), not extra water. To the best of the authors' knowledge, no peer reviewed publications exist that quantify the required volume or the minimum quality of the recycled water.

A dairy flushing will use 3 to 5 gallons of water per square foot of floor space per day. A typical dairy layout has approximately 48 square feet of alley space per cow. Actual square footage may be larger, depending on cross alleys, or smaller if 3-row pens are used. This suggests that between 150 and 250 gallons or 1,200 to 2,000 lb of water are required for flushing alleys each day per cow. If 50 lb of sand are used per stall, then 24 to 40 lb of water is required per lb of sand removed. The ratio of flush water to excreted manure ranges from 8:1 to 14:1.

Recycled water contains nutrients and solids that tend to increase over time if no dilution or make-up water is added to the system. Total solids content in the water influences quality of recovered sand. As total solids in recycled water increase, sand will separate but

may contain excess organic matter that is not suitable for reuse. The authors recommend that the recycled water have a maximum of 2 to 4% total solids, with less being better. No intensive field studies have been published, however, that quantify the impact of water quality versus reclaimed sand quality. Manure as excreted contains 12 to 13% total solids. Including the parlor wash, water reduces total solids content to < 10% in most cases. Total solids of the final stream may be reduced if solid separators are used between the sand separation phase and the liquid storage. The objective of this study is to develop a procedure to estimate the total supplemental or make-up water necessary to reach a desired solid content in the recycled water.

### Procedures

A spreadsheet model was developed to determine the additional water requirements. The weight of the dry matter weight was determined by:

Eq. 1  

$$TS_{dm} = M_{excreted} \times (100 - MC_{initial}) / 100,$$

where:  
 $TS_{dm}$  = total solids (dry matter basis, lb),  
 $M_{excreted}$  = total manure excreted (lb), and  
 $MC_{initial}$  = initial moisture content of the solids (% wb).

Quantity of material removed by the separator was calculated by:

Eq. 2  

$$Wgt_{separator} = (TS_{dm} \times S_{eff} / 100) \times 100 / (100 - MC_{solids}),$$

where:  
 $Wgt_{separator}$  = total weight of the material removed (lbs),

$S_{eff}$  = the separator removal efficiency (%), and  
 $MC_{solids}$  = final moisture content of the removed solids (% wb).

Final weight of the material is calculated based on the desired solid content of the waste stream entering a containment structure.

Eq. 3  

$$Wgt = Wgt_{separator} \times (100 - MC_{solids}) / (D_{solids})$$

where:  
 $Wgt$  = final weight of the material entering the structure (lb), and  
 $D_{solids}$  = desired solid content of the material in the structure (%).

Additional water requirements may then be determined by using:

Eq. 4  

$$Wgt_{water} = Wgt - Wgt_{separator}$$

where:  
 $Wgt_{water}$  = weight of the extra water required (lbs).

The Excel spreadsheet model assumed a fixed manure production of 140 lb of manure/day per cow at a moisture content of 87.5%. Separator efficiencies evaluated ranged from 10 to 80%. Final moisture content of the separated material was varied from 50 to 80%. Separator efficiency is defined as the percentage of total solids removed as determined by total input solids and output solids. Total solid removal is based on a dry matter basis and includes both dissolved and non dissolved solids. Separated material represents the portion of the input waste stream separated by the separator and is partitioned or stored somewhere other than the liquid containment structure.

## Results

Figure 1 shows total weight reduction as a function of solid separator efficiency and moisture content of the separated solids. The graph shows that if separator efficiency is 20% or less, that regardless of moisture content, the final weight reduction will be < 20 lb. Figure 2 illustrates the percentage weight reduction as a function of separator efficiency and separated solids moisture content. Using the 20% removal efficiency, total weight reduction of the manure stream entering the liquid containment structure is < 15%. These graphs illustrate that even though material is being removed by a separator, there is still a large percentage of the solids and liquids fraction entering the liquid containment structures.

Tables 1, 2, 3 and 4 show the supplemental, or make-up, water required to reach a desired solids content level of 2, 3, 4, and 6%, respectively, in the containment structure. Make-up water necessary to maintain the total solids in the recycled flush water at 2% is shown in Table 1. Solid separator efficiency affects extra water requirements more than final moisture content of separated solids. Because the dry matter mass of the removed solids is fairly low (Figure 1), less moisture is necessary to reach the final weight. Additional water requirements range from 79 to 13 gallons, depending on the separator efficiency. An increase in supplemental water is required as separator efficiency decreases or moisture content increases. If a two- or three-stage lagoon system is used for solid separation rather than a solid to liquid separation process, then the water requirements are 89, 53, 36, and 18 gallons/cow per day to reach a total solids content of 2, 3, 4, and 6%, respectively.

Impact of desired total solids in the recycled water may be seen by comparing Tables 1 and 4. This is illustrated by comparing the additional water requirements assuming 50% total solids removed and a moisture content of 70%. Table 1 indicates that 39 gallons/cow

per day is required, and Table 4 shows only 4 gallons/cow per day are required. Table 4 is representative of the situation on many dairies in which the only supplemental or make up water added to the system is through rain water on to the surface of the containment structure and parlor wash water. Additional water requirements are 15 gallons or less, but the recycled water will contain 6% total solids, and high quality sand may not be recovered.

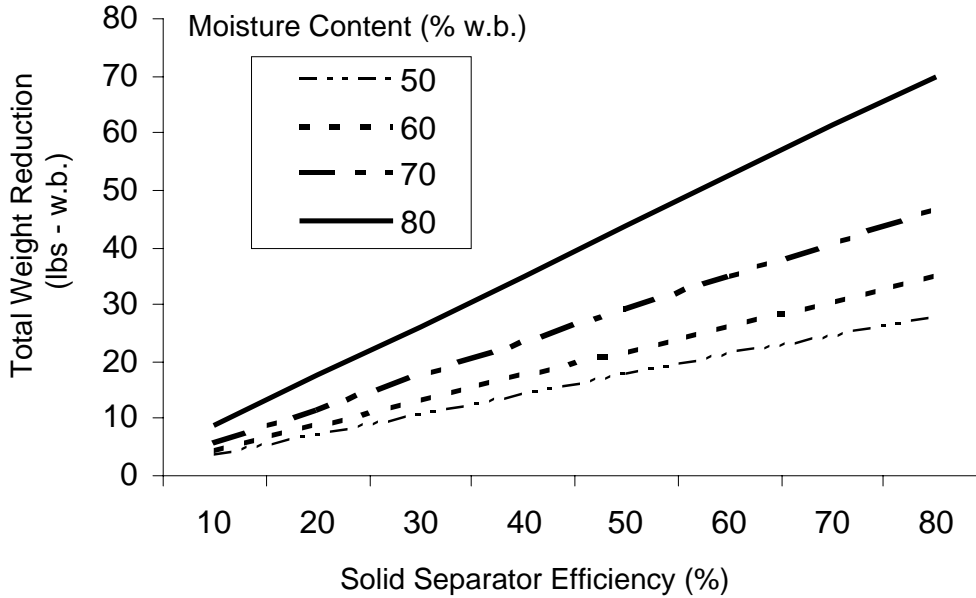
Many separators have a reported efficiency of 20 to 50%. Twenty to 40 gallons of supplemental, or make-up, water/cow per day is required if 3% total solids in the recycled water is desired (Table 3). Sources of this supplemental water may come from rainwater onto the containment structure surface, milk parlor wash water or clean up water, extraneous drainage, or make-up water from a freshwater pond or groundwater.

## Conclusions

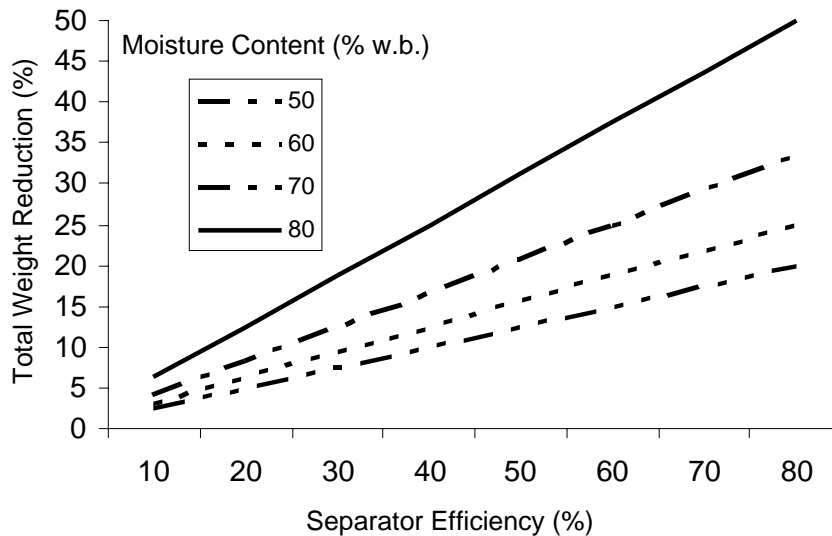
Successful reclamation of sand requires supplemental water to adequately separate the organic and inorganic matter. Separator efficiency and moisture content of the separated solids influence the make-up water requirements on dairies seeking to use recycled water for sand reclamation. Additional water requirements range from 0 to 79 gallons/cow per day depending on the solid to liquid separation process before a containment structure. If supplemental water is required, then the design engineer must determine if there is adequate water available for recycling. Daily disposal cost of this extra water may increase variable costs up to \$0.79/cow per day if the separation process is inefficient, assuming an application cost of \$0.01 per gallon. Operating costs may be reduced by purchasing sand for bedding rather than handling extra water requirements for recycling sand if the solid to liquid separation process is inefficient or if irrigation is not available to handle the additional water. Supplemental water requirements

are influenced by the efficiency of the solid to liquid separation process and the acceptable percentage of total solids content in the con-

tainment structure. Final moisture content of the separated solids has only marginal impact on the daily disposal cost.



**Figure 1. Impact of Solid Separator Efficiency and Final Moisture Content of the Separated Material on the Weight of Material Removed Assuming a Cow Excretes 140 lb of Manure per Day.**



**Figure 2. Impact of Solid Separator Efficiency and Final Moisture Content of the Separated Material on the Volume Reduction Assuming a Cow Excretes 140 lb of Manure per Day.**

**Table 1. Supplemental Water (gallons) Required per Cow per Day to Maintain Total Solids in a Containment Structure at 2% Based on Total Solids Removed and Final Moisture Content of the Separated Material Assuming the Cow Excretes 140 lb of Manure per Day**

Total solids removed, %	Final moisture content of solids, % wet bulb			
	50	60	70	80
10	78	79	79	79
20	68	69	69	70
30	58	59	59	60
40	48	48	49	52
50	38	38	39	41
60	28	28	30	32
70	18	18	20	22
80	8	8	10	13

**Table 2. Supplemental Water (gallons) Required per Cow per Day to Maintain Total Solids in a Containment Structure at 3% Based on Total Solids Removed and Final Moisture Content of the Separated Material Assuming the Cow Excretes 140 lb of Manure per Day**

Total solids removed, %	Final moisture content of solids, % wet bulb			
	50	60	70	80
10	47	47	47	47
20	40	40	41	41
30	34	34	34	35
40	27	27	28	30
50	20	21	22	24
60	14	14	15	18
70	7	8	9	12
80	1	1	3	6

**Table 3. Supplemental Water (gallons) Required per Cow per Day to Maintain Total Solids in a Containment Structure at 4% Based on Total Solids Removed and Final Moisture Content of the Separated Material Assuming the Cow Excretes 140 lb of Manure per Day**

Total solids removed, %	Final moisture content of solids, % wet bulb			
	50	60	70	80
10	31	31	31	32
20	26	26	27	27
30	21	22	22	23
40	16	17	18	19
50	12	12	13	15
60	7	7	8	11
70	2	3	4	6
80	0	0	0	2

**Table 4. Supplemental Water (gallons) Required per Cow per Day to Maintain Total Solids in a Containment Structure at 6% Based on Total Solids Removed and Final Moisture Content of the Separated Material Assuming the Cow Excretes 140 lb of Manure per Day**

Total solids removed, %	Final moisture content of solids, % wet bulb			
	50	60	70	80
10	15	15	15	16
20	12	12	13	13
30	9	9	10	11
40	6	6	7	8
50	3	3	4	6
60	0	0	0	0
70	0	0	0	0
80	0	0	0	0



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## ABILITY OF LOW PROFILE CROSS-VENTILATED FREESTALL BARNs TO IMPROVE ENVIRONMENTAL CONDITIONS FOR DAIRY CATTLE

J. F. Smith, J. P. Harner, and M. J. Brouk

### Summary

Recently, there has been interest in constructing mechanical ventilation with evaporative pads to improve the environmental conditions for cows during periods of heat stress. Low profile cross-ventilated freestalls with evaporative pads (LPCV) have become a popular system. The purpose of this study was to evaluate how well these LPCV systems improve the temperature-humidity index (THI) under different ambient conditions. As ambient humidity increases, ability of the LPCV to reduce THI is decreased. Producers wishing to construct LPCV barns should carefully evaluate the climate in which they want to construct LPCV structures.

(Key words: THI, heat stress, cross ventilation.)

### Introduction

Recently, producers have used cross-ventilation with evaporative pads to cool the air around the cow. As water is evaporated into the air, temperature will drop, and humidity will increase. Expected changes in THI, under different environmental conditions, using evaporative cooling, is presented in Figures 1 and 2. As humidity increases, it becomes more difficult to change the environment in which the cow is housed. It is important to have realistic expectations about the ability of these systems to change the environmental conditions in which they will be operated.

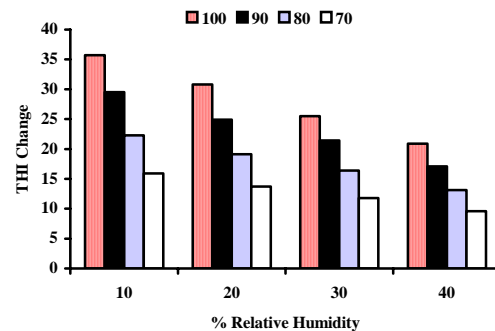


Figure 1. Potential THI Change in Response to Water Evaporation at 100°, 90°, 80°, and 70°F in a Low Relative Humidity Environment (Adapted from ASHRAE Handbook, 1993).

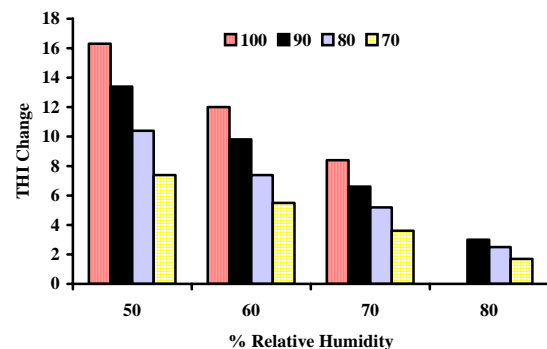


Figure 2. Potential THI Change in Response to Water Evaporation at 100°, 90°, 80°, and 70°F in a High Relative Humidity Environment (Adapted from ASHRAE Handbook, 1993).

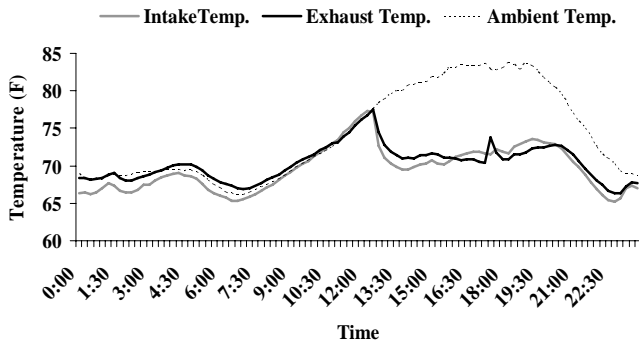
## Procedures

During the summer of 2006, data loggers were used to evaluate an 8-row, low-profile, cross-ventilated, freestall barn with evaporating pads to determine the ability of this system to reduce heat stress under different environmental conditions. The facility evaluated was 210 feet wide by 420 feet long, with a side-wall height of 13 feet, and a roof pitch of 0.5 feet in 12 feet. Two data loggers were installed to monitor ambient, barn intake, and barn exhaust temperature and percent relative humidity every 15 minutes. The THI was calculated for ambient, barn intake, and barn exhaust locations.

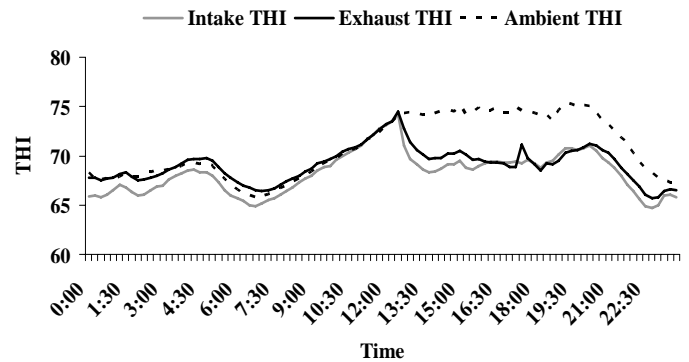
## Results and Discussion

Temperature data collected in this study demonstrates the limitation of the LPCV sys-

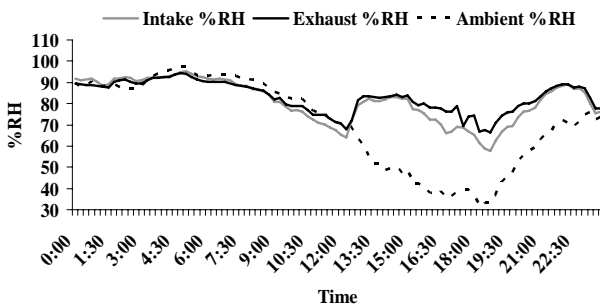
tem to improve the environment inside the structure during periods of high humidity. Ambient barn intake and barn exhaust temperature, relative humidity, and THI for 4 different days (July 1, 4, 26, and 29, 2006) with different ambient conditions are presented in Figures 3 through 14. These figures demonstrate that as ambient humidity increases, ability to reduce temperature with evaporative cooling (evaporative pads) and cross ventilation is compromised. Individual climates should be evaluated to set realistic expectations on how well the LPCV system will improve environmental conditions. Further research is needed to investigate the combination of soaker and evaporative cooling to reduce potential heat stress during periods of high relative humidity and high temperatures.



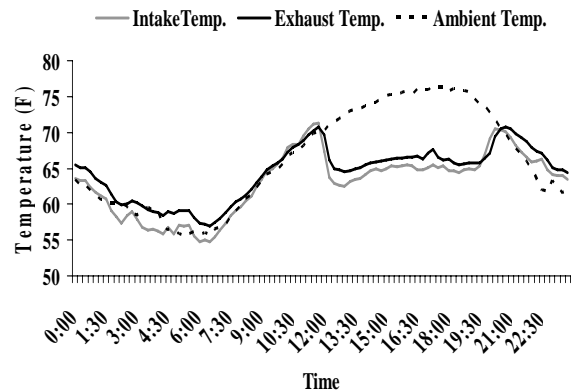
**Figure 3. Typical Day Temperatures (July 1, 2006).**



**Figure 5. Typical Day THI (July 1, 2006).**



**Figure 4. Typical Day Relative Humidity (July 1, 2006).**



**Figure 6. Typical Cool Summer Day Temperatures (July 4, 2006).**

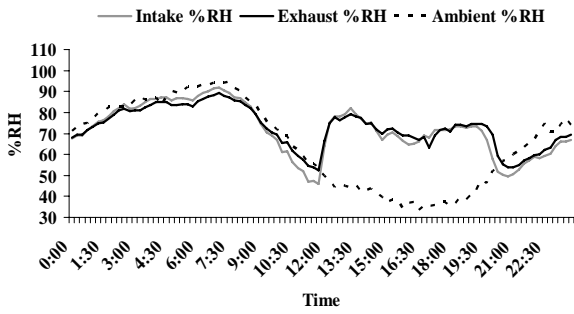


Figure 7. Typical Cool Summer Day Relative Humidity (July 4, 2006).

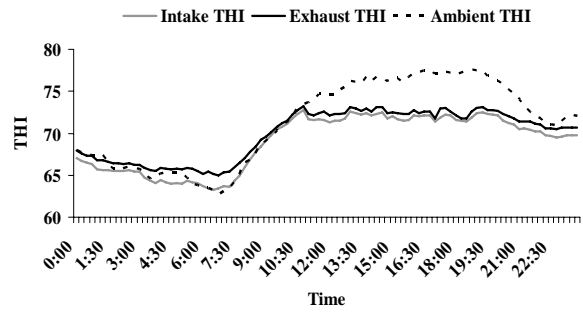


Figure 11. Typical Low Humidity Day THI (July 26, 2006).

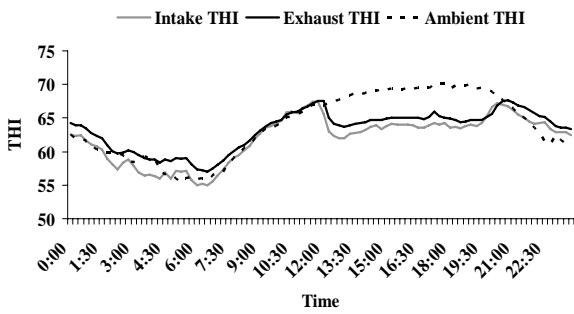


Figure 8. Typical Cool Summer Day THI (July 4, 2006).

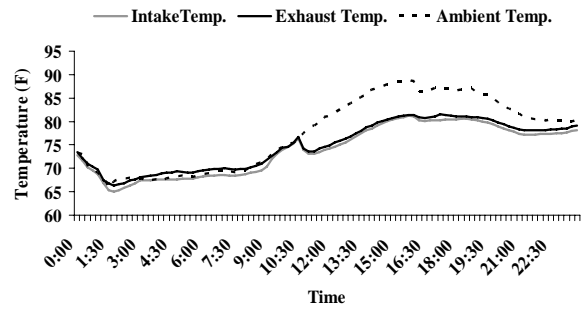


Figure 12. Typical Very Humid Day Temperatures (July 29, 2006).

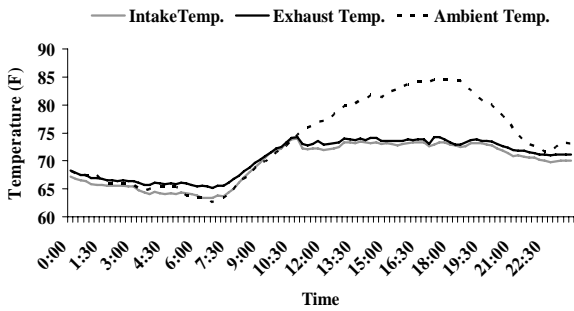


Figure 9. Typical Low Humidity Day Temperatures (July 26, 2006).

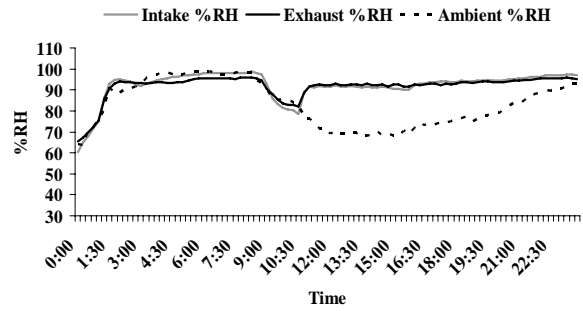


Figure 13. Typical Very Humid Day Relative Humidity (July 29, 2006).

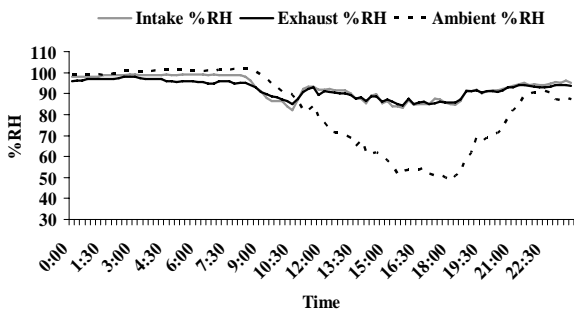


Figure 10. Typical Low Humidity Day Relative Humidity (July 26, 2006).

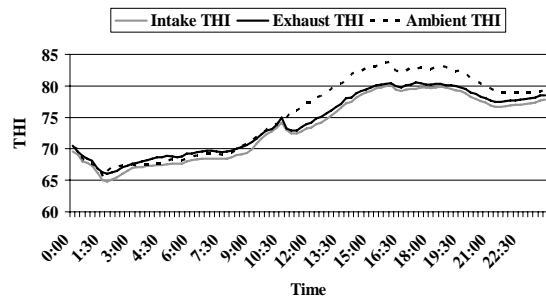


Figure 14. Typical Very Humid Day THI (July 29, 2006).

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**INDEX OF KEY WORDS**

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Diamond V, Cedar Rapids, IA	Meier Dairy of Palmer, Inc., Palmer, KS
Elanco Animal Health, Greenfield, IN	Merial Limited, Iselin, NJ
Environmental Health Protection Agency, Washington, D.C.	Monsanto Company, St. Louis, MO
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## **BIOLOGICAL VARIABILITY AND CHANCES OF ERROR**

Variability among individual animals in an experiment leads to problems in interpreting the results. Although the cattle on treatment X may have produced more milk than those on treatment Y, variability within treatments may indicate that the differences in production between X and Y were not the result of the treatment alone. Statistical analysis allows us to calculate the probability that such differences are from treatment rather than from chance.

In some of the articles herein, you will see the notation "P<.05". That means the probability of the differences resulting from chance is less than 5%. If two averages are said to be "significantly different," the probability is less than 5% that the difference is from chance, or the probability exceeds 95% that the difference resulted from the treatment applied.

Some papers report correlations or measures of the relationship between traits. The relationship may be positive (both traits tend to get larger or smaller together) or negative (as one trait gets larger, the other gets smaller). A perfect correlation is one (+1 or -1). If there is no relationship, the correlation is zero.

In other papers, you may see an average given as  $2.5 \pm 0.1$ . The 2.5 is the average; 0.1 is the "standard error." The standard error is calculated to be 68% certain that the real average (with an unlimited number of animals) would fall within one standard error from the average, in this case between 2.4 and 2.6.

Using many animals per treatment, replicating treatments several times, and using uniform animals increase the probability of finding real differences when they exist. Statistical analysis allows more valid interpretation of the results, regardless of the number of animals. In all the research reported herein, statistical analyses are included to increase the confidence you can place in the results.

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