

# ANNUAL REPORT OF REGIONAL RESEARCH PROJECT NC-1006

October 1, 2005 to September 30, 2006

**Project Title:** Methods to Increase Reproductive Efficiency in Cattle

## **Accomplishments:**

### **Collaborative studies:**

**Cooperative Dairy Project** (Station participants: Indiana, Kansas, Michigan, Missouri, Minnesota, and Wisconsin)

### **Hypotheses**

1. Noncycling dairy cows can be identified by heat-detection patches (Kamar), or 1 or 2 transrectal ultrasonographic ovarian exams.
2. Noncycling dairy cows have improved pregnancy rates after treatment with progesterone and GnRH.
3. Altering timing of AI relative to an Ovsynch-like protocol will maximize pregnancy rates in noncycling and cycling dairy cows.

### **Objectives**

1. To determine whether noncycling dairy cows:
  - a. Can be identified accurately by use of a heat-detection patch (Kamar).
  - b. Can be identified accurately by 1 (10 days before timed AI or TAI) or 2 (3 and 10 days before TAI) ultrasonographic exams.
  - c. Pregnancy rates can be improved by insertion of an intravaginal progesterone-releasing insert (CIDR) in conjunction with an Presynch + Ovsynch ovulation-synchronization breeding protocol.
  - d. Pregnancy rates can be improved by altering timing of AI to occur concurrent with GnRH or 24 h after GnRH injection.
  - e. Pregnancy loss may be reduced by previous exposure to the CIDR or altered timing of TAI.
2. To ascertain whether pregnancy rates and pregnancy loss of cycling dairy cows are improved by altering timing of AI to occur concurrent with GnRH or 24 h after GnRH injection.

### **Experimental Approach**

All cows will receive 2 injections of PGF<sub>2α</sub> 14 days apart, with the second injection given 14 days before initiating the Ovsynch protocol [first Presynch injections should begin between 30 and 40 days postpartum so first services (TAI) occur from 67-68 to 80-81 days].

**Noncycling cows.** One-half of the noncycling cows verified by ultrasound ovarian exam on day 28 of the protocol (first injection of GnRH) will be treated for 7 days with an intravaginal progesterone-releasing insert (CIDR) during the first 7 days of the Ovsynch protocol and one-half will serve as controls without the CIDR insert. Within each group of noncycling cows

(CIDR vs. no CIDR), cows will be re-randomized to be inseminated: 1) concurrently with the injection of GnRH at 48 h after the PGF<sub>2α</sub> injection of Ovsynch; or 2) 24 h after the injection of GnRH (GnRH given at 48 h after PGF<sub>2α</sub> of Ovsynch). Thus, 4 treatments of noncycling cows will be formed: GnRH48 + TAI48 + CIDR; GnRH48 + TAI48 + No CIDR; GnRH48 + TAI72 + CIDR; and GnRH48 + TAI72 + No CIDR.

**Control cows.** Control cycling cows will be treated with the Ovsynch protocol, but inseminated (as noncycling cows): 1) concurrently with the injection of GnRH at 48 h after the PGF<sub>2α</sub> injection of Ovsynch; or 2) 24 h after the injection of GnRH (GnRH given at 48 h after PGF<sub>2α</sub> of Ovsynch). Thus, 2 treatments of control cycling cows will be formed (GnRH48+TAI48 and GnRH48+TAI72).

## Results

Kamar patches in place during 4 wk before the initiation of the Ovsynch protocol overestimated the proportion of cows, whose status was later verified by serum concentrations of progesterone, which have had previous estrous cycles (gold standard is based on 3 blood samples collected before the 2 Presynch injections and the first GnRH injection of Ovsynch). Ultrasound underestimated the proportion of cycling cows probably by classifying proestrous, estrous (not detected), and metestrous cows not having a CL as noncycling or anestrous cows. For control cows (those classified as cycling by ultrasound and serum progesterone), Kamar overestimated cycling activity by ultrasound and slightly underestimated cycling activity in cows verified by serum progesterone.

Pregnancy rates at day 33, day 61, and pregnancy losses did not differ ( $P > 0.20$ ) between cows classified as anestrous that received a CIDR insert compared with those anestrous cows that did not received a CIDR. Although pregnancy rates at days 33 and 61 were numerically greater for control cycling cows inseminated 24 h after GnRH, no differences were detected ( $P > 0.020$ ). Further, timing of AI in anestrous cows did not seem to differ, but cows receiving a CIDR insert always had numerically better fertility. Cows classified as anestrous had reduced ( $P < 0.01$ ) pregnancy rates at days 33 and 61 compared with cycling control cows. Pregnancy losses were numerically smaller in those cows previously treated with a CIDR insert, but did not differ from those losses detected in previously cycling control cows. Pregnancy rate at day 31 is shown when categorized by cycling status that was determined by serum progesterone.

## Accomplishments:

1. In lactating dairy cows whose estrous cycles were presynchronized before applying a TAI protocol (Ovsynch), delaying the PGF<sub>2α</sub> injection of Ovsynch by 24 h, had little effect on outcomes, but delaying by 48 h might reduce conception rates.
2. In lactating dairy cows, Kamar patches in place during 4 wk before initiating a TAI protocol (Ovsynch) overestimated previous estrual activity in cows subsequently classified as anestrus. Kamar overestimated previous estrual activity compared with 1 ultrasound exam conducted before the first GnRH injection of Ovsynch. Kamar slightly underestimated previous estrual activity in cows later classified as cycling. Fertility (pregnancy rate or pregnancy loss) was not improved significantly by addition of the CIDR insert to cows identified as anestrus. Cows previously identified as anestrus, however, had reduced pregnancy rates at days 33 and 61 (regardless of whether they received a CIDR insert) compared with control cows classified as cycling.

## **Cooperative Beef Project** (Station participants: Illinois, Kansas, and Minnesota)

### **Influence of a CIDR insert after a fixed-time AI on pregnancy rates and return to estrus of nonpregnant cows.**

We determined whether resynchronization of an ovulatory estrus could be accomplished in nonpregnant cows without compromising pregnancy in cows pregnant from a previous synchronized estrus or to those inseminated to the resynchronized estrus. Ovulation was synchronized in 937 suckled beef cows at 6 locations using a CO-Synch + CIDR protocol (a 100- $\mu$ g injection of GnRH at the time of CIDR insertion, followed in 7 d by a 25-mg injection of PGF<sub>2 $\alpha$</sub>  at CIDR removal. At 60 h after PGF<sub>2 $\alpha$</sub> , cows received a fixed-time AI [TAI] plus a second injection of GnRH. After initial TAI cows were assigned randomly to 4 treatments; 1) untreated (control; n = 237); 2) CIDR inserted 5 d after TAI and removed 14 d after TAI (CIDR5-14; n = 234); 3) CIDR inserted 14 d after TAI and removed 21 d after TAI (CIDR14-21; n = 232); or 4) CIDR inserted 5 d after TAI and removed 14 d after TAI and then a new CIDR inserted at 14 d and removed 21 d after TAI (CIDR5-21; n = 234). After TAI, cows were observed twice daily until 25 d after TAI for estrus and inseminated according to the AM-PM rule. Pregnancy was determined at 29 and 59 d after TAI to determine conception to first- and second-service AI. Pregnancy rates to TAI were similar for control (55%), CIDR5-14 (54%), CIDR14-21 (48%), and CIDR5-21 (53%). A greater proportion of nonpregnant cows were resynchronized during a 2-d peak period in the CIDR5-21 (76/109, 70%) and CIDR14-21 (77/119, 65%) than controls (44/106, 42%) and CIDR5-14 (39/109, 36%) cows. Although overall pregnancy rates after second AI service were similar, conception rates of nonpregnant cows detected in estrus and inseminated seemed to be compromised ( $P < 0.05$ ) in CIDR5-21 (41/76, 54%) and CIDR14-21 (71/77, 53%) compared with CIDR5-14 (28/39, 72%) cows, whereas controls (29/44, 66%) were intermediate. Insertion of a CIDR 5 d after a TAI did not compromise or enhance pregnancy rates to TAI, however, conception rates were compromised in nonpregnant cows that were resynchronized with a CIDR from d 5 or 14 until 21 d after TAI.

### **Impact Statement:**

- Our collaborative research has the potential to increase reproductive efficiency in dairy and beef cattle, which will prevent lost income associated with reproductive losses for the producer and increase profitability. Improved procedures for estrous synchronization in beef heifers and lactating dairy cows will increase farm income by increasing milk production, through reduced days open and genetic improvement (widespread use of artificial insemination). The increase in milk production is conservatively valued at \$100/cow.

## Individual Station Reports:

*Minnesota:*

### **Experiment 1: Effect of hCG on ovarian structure dynamics and concentrations of progesterone in non-cycling Angus heifers.**

We determined the effect of administering 1,000 IU or 500 IU hCG on subsequent ovarian structure dynamics and concentrations of progesterone in purebred Angus heifers. Blood samples were collected 14 and 4 d before treatment to determine estrous cyclicity status and heifers with concentrations of progesterone in serum  $\geq 1$  ng/ml at one or both time points were defined as pubertal and removed from the experimental pool. The remaining heifers (n = 47) were stratified by age and weight and randomly assigned to one of three treatments: 1) heifers received a 100  $\mu$ g injection of GnRH (n = 16); 2) heifers received 1000 IU hCG (n = 16); and 3) heifers received 500 IU hCG (n = 15). The ovaries of each heifer were examined daily and mapped by transrectal ultrasonography to monitor ovarian structure dynamics beginning one day before treatment was administered and continuing until day nine after treatment. In addition, blood samples were collected daily starting the day before treatment until day nine after treatment. Concentrations of progesterone in serum were determined by RIA.

### **Experiment 2: Incorporation of hCG into a timed-AI estrous synchronization protocol for beef heifers.**

We determined the effect of administering hCG 14 d prior to initiation of the Co-Synch protocol and whether replacing GnRH with hCG at CIDR insertion would enhance pregnancy rates in the CO-Synch protocol. Using a 2 x 2 factorial design of treatments five hundred forty seven replacement beef heifers were assigned to one of four treatments (Figure 1): 1) heifers received a 100  $\mu$ g injection of GnRH at CIDR insertion (d -7) and a 25 mg injection of PGF at CIDR removal (d 0), followed in 54 hr by a fixed-time AI (TAI) with a second injection of GnRH (Control GnRH; CG; n = 160); 2) CG but the first injection of GnRH was replaced with a 1,000 IU of human chorionic gonadotropin (hCG; CH; n = 158); 3) CG, plus heifers received a 1,000 IU injection of hCG 14 d prior to CIDR insertion (HG; n = 116); and 4) CH, plus heifers received a 1,000 IU injection of hCG 14 d prior to CIDR insertion (HH; n = 113). Blood samples were collected on d -31, -21, -14, -7, 0, 2 to harvest serum for later analysis of concentrations of progesterone. Progesterone was used to determine cycling status and response to estrous synchronization. Transrectal ultrasonography was performed at CIDR insertion and CIDR removal to determine response of ovarian structures to treatment. Pregnancy was diagnosed by transrectal ultrasonography on d 33 after TAI. Overall there was a difference in pregnancy rates among heifers originating from four separate locations with a range from 16 to 40%. Cycling prior to initiation of treatments did not affect overall pregnancy rates and heifers receiving a 1,000 IU injection of hCG 14 d prior to initiation of estrous synchronization did not have an increase in pregnancy rates. However, substituting GnRH with a 1,000 IU injection of hCG at CIDR insertion resulted in reduced pregnancy rates. Overall there were no statistical differences among the four treatments (37, 30, 46, and 28 % for CG, CH, HG, and HH treatments, respectively). We conclude that replacement of the first injection of GnRH of the CO-Synch + CIDR protocol with a 1,000 IU injection of hCG appear to result in reduced pregnancy rates and is not a suitable alternative to GnRH.

### **Experiment 3: Effects of estrous synchronization with a CIDR prior to the breeding in bull-breeding herds on pregnancy rates.**

We determined whether insertion of a CIDR prior to the breeding season enhanced pregnancy rates and altered the date of conception in suckled beef cows mated naturally. One thousand seven hundred and fifty suckled beef cows from thirteen locations were randomly assigned to one of two treatments: 1) cows received a CIDR 7 d prior to the breeding season for 7 d (CIDR; n = 866); 2) cows received no treatment (Control; n = 884). On the first day of the breeding season bulls were introduced to the herd at a rate of 15 to 25 cows per yearling bull or 20 to 30 cows per mature bull. Cows were evaluated by transrectal ultrasonography for pregnancy at 56 d and 120 d after initiation of the breeding season to determine pregnancy status and date of conception. Overall pregnancy rates ranged from 59.3 to 98.9% among the 13 locations. Pregnancy rates within the first 30 days of the breeding season were similar between CIDR (64.4%) and Control (64.7%), and overall pregnancy rates were similar between CIDR (89.7%) and Control (89.6%). The average day of conception after initiation of the breeding season was shorter ( $P < 0.05$ ) for CIDR ( $20.1 \pm 0.8$  d) compared to Control cows ( $23.2 \pm 0.8$  d). Of cows conceiving during the breeding season, more ( $P < 0.05$ ) CIDR cows (43%) conceived during the first ten days of the breeding season than Control cows (35%). Body condition score and parity did not affect pregnancy rates or days to conception, whereas pregnancy rates and days to conception were affected ( $P < 0.01$ ) by location and days postpartum. Days to conception were similar between treatments for cows calving within 50 d of initiation of the breeding season ( $28.2 \pm 1.0$  d), whereas cows calving earlier in the calving season treated with a CIDR ( $16.1 \pm 0.9$  d) conceived earlier ( $P < 0.05$ ) than Control cows ( $20.7 \pm 0.9$  d). We concluded that insertion of a CIDR prior to the breeding season failed to increase overall pregnancy rates, but did influence the average day of conception in earlier calving cows.

### **Impact Statements**

- Reports have indicated that the use of artificial insemination in the beef industry remains low (between 5 and 8% of producers) because of factors such as labor, time, and convenience. We have developed two short (less than 10 days) estrous synchronization systems that can be utilized by producers with 60 to 90 % pregnancy rates. One of those systems (CO-Synch+CIDR) is a fixed-time AI system that requires no detection of estrus, thus providing a protocol that reduces time associated with detection of estrus in beef herds.
- Developing methods to enhance response of follicles to fixed-time AI protocols is critical to enhancing overall pregnancy rates to those protocols. Therefore, the potential for the use of hCG in heifer estrous synchronization protocols could enhance fertility to heifers exposed to fixed-time AI and result in a greater proportion of AI sired calves. In addition, improvement in fertility will generate more interest by beef producers to utilize fixed-time AI estrous synchronization systems, thus enhancing the overall use of AI.
- Methods of concentrating the calving season and developing more uniform calf crops enhance the income generated for calves and also increase marketing opportunities of those calves. Therefore, altering the calving season through estrous synchronization of bull breeding herds has the potential to impact 90% of beef producers that do not utilize AI to alter their calving distribution.
- Synchronization of the return estrus in non-pregnant cows has application in herds that utilize a second AI. Concentrating the percentage of non-pregnant cows returning to estrus within a short window reduce the time associated with daily estrus detection. Therefore, we have

demonstrated that cows can be effectively resynchronized with a CIDR and inseminated within a short window of time after CIDR removal.

*Kansas:*

### **Experiment 1: Delaying injection of prostaglandin F<sub>2α</sub> in an Ovsynch protocol.**

Our objective was to determine whether delaying PGF<sub>2α</sub> injection by 24 or 48 h after the first GnRH injection in an Ovsynch protocol (from a standard 7 d) altered ovarian characteristics in lactating dairy cows. Beginning 9 d after removal of a progesterone (P4)-releasing, controlled internal drug release (CIDR) insert and injection of PGF<sub>2α</sub> (d 6.4 of the estrous cycle; 65 ± 2 d in milk), 36 Holsteins (average body weight = 707 ± 12 kg and BCS = 2.3 ± 0.1) were administered 100 µg of GnRH and assigned randomly to receive treatment injections of PGF<sub>2α</sub> 7, 8, or 9 d later. Timed artificial insemination was performed at 48 h after PGF<sub>2α</sub>, at which time a second injection of GnRH was administered (91 ± 2 d). Ovarian structures were mapped by ultrasonography on d 0 (first GnRH injection); on d 2 to determine responses to the first GnRH injection; at PGF<sub>2α</sub> injection; and daily thereafter through 72 after PGF<sub>2α</sub>. Blood was collected on d 0, 2, at PGF<sub>2α</sub> injection, and at 24 and 48 h after PGF<sub>2α</sub> to monitor serum changes in estradiol-17β (E2-17β) and progesterone (P4). On the basis of serum P4 and ovarian exams, 2 cows were eliminated because of anestrus and their failure to ovulate a follicle in response to the first GnRH injection. Two other cows in which luteolysis failed to occur after PGF<sub>2α</sub> treatment also were eliminated. Final numbers of cows per treatment were: 7 d (n = 13), 8 d (n = 9), and 9 d (n = 10). Twenty-nine of 32 cows ovulated (90.6%) in response to the first GnRH injection. Of those cows not ovulating in response to the first GnRH injection, 2 had 1 original corpus luteum and 1 had 2 original corpora lutea. Despite a 24- or 48-h delay between first GnRH and PGF<sub>2α</sub> injections, diameter (mm) and volume (mm<sup>3</sup>) of the ovulatory follicle did not differ among treatments (Table 1). In all 32 cows, at least 1 follicle ovulated after treatment, but ovulation rates did not differ (Table 1). Serum concentrations of E2-17β did not differ among treatments. Two cows in the 7-d and 2 cows in the 8-d treatments were inseminated 24 h late and were excluded before assessing conception rates (Table 1). We conclude that in cows whose estrous cycles were presynchronized, delaying the PGF<sub>2α</sub> injection of Ovsynch by 24 h, had little effect on outcomes, but delaying by 48 h might reduce conception rates.

### **Experiment 2: Post-AI interventions in lactating dairy cattle. Ovarian responses, conception rates and pregnancy survival in response to GnRH, human chorionic gonadotropin (hCG), and exogenous progesterone (CIDR).**

We hypothesized that increasing concentrations of progesterone (P4) after artificial insemination (AI) would increase fertility. Our objective was to assess changes in ovarian structures, incidence of ovulation, and change in serum P4 in response to GnRH, human chorionic gonadotropin (hCG), or exogenous P4 (controlled internal drug release; CIDR insert) treatment beginning 4 to 9 d after AI (d 0) and again 7 d later (Exp. 1). Blood was collected from 753 cows in 3 herds on d 0 and 7. Ovaries of 162 cows were scanned and mapped to confirm the presence a corpus luteum (CL) and cows were assigned randomly to serve as controls (n = 41) or to receive a CIDR insert for 7 d (n = 41), 100 µg of GnRH (n = 40), or 3,300 IU of hCG (n = 40). More cows were induced to ovulate in response to GnRH (60%) and hCG (78%) compared with controls (2.4%). Compared with controls, cows treated with GnRH or hCG had more induced CL (d 7) and more total CL (d 7), but serum P4 was increased only in response to hCG. Largest

follicle diameters on d 7 were less after GnRH and hCG, but total follicular volume on d 7 was reduced by GnRH, hCG, and CIDR, compared with that of controls. Volume of the original luteal structures was increased by hCG, but tended to be reduced by CIDR and GnRH compared with luteal volume in controls. Total CL volume was increased by hCG, but reduced by CIDR, compared with CL volume of controls. Conception rates and pregnancy survival (Exp. 2) were assessed in response to the same treatments described in Exp. 1: controls (n = 708), CIDR (n = 711), GnRH (n = 719), and hCG (n = 714). Results are in Tables 3 and 4. Tendencies for interactions of treatment × herd and treatment × lactation group were detected, but no 3-way interactions were found. Treatment with hCG increased conception rates in second-lactation cows. The CIDR tended to increase, and hCG increased, conception rates in 2 herds, whereas the CIDR decreased conception rates in 1 herd. Pregnancy survival was reduced by GnRH, compared with that in controls. We concluded that GnRH and hCG effectively induced ovulation, and increased number of CL, but only increased serum P4 in hCG-treated cows. Further, treatment with the CIDR or hCG increased conception rates, but only in some herds.

### **Impact Statement**

- Applying an intravaginal progesterone-releasing insert for 7 d beginning at 6 to 8 days after insemination or injecting hCG once during the same period resulted in an 4.5 to 5.3 percentage improvement in pregnancy rate. In other words, for every 100 cows inseminated, 4 to 5 more cows would become pregnant each week, if treated as described. When a cow that produces 100 pounds of milk per day (valued from \$12/lb) and fails to conceive, an opportunity is lost for a minimum of 3 to 7 weeks. That loss is valued at \$4 per day. Therefore, improving pregnancy rates would reduce lost income of \$81 to 196 per cow. For a 100-cow dairy, this loss would range from \$8,100 to 19,600 per year.

*Purdue:*

### **Experiment 1. Murine microarray studies.**

Poly A+ RNA was extracted using the Dynal Bead Kit. 5 ul (1/10 RNA) of each sample was removed for G3PDH amplification to confirm the presence of mRNA and run a mock amplification to determine if genomic DNA was present in the samples. The remainder of the samples were dried. All samples produced a G3PDH band after PCR amplification and gel analysis and did not produce a mock band indicating that the samples did not contain genomic DNA contamination. Dr. Bidwell performed the two-cycle amplification using the Affymetrix GeneChip Two-Cycle cDNA Synthesis Kit. The core facility was responsible for only the hybridizations. Two samples did not contain enough cDNA (in vivo matured d26 and immature d20) and two samples did not produce good cDNA (immature d56 and in vitro matured d56) for hybridization. Twenty Affymetrix microarrays have been ordered, and we will hybridize chips later this month.

Work on RT-PCR has begun to determine the fewest number of oocytes that are required for confirmation of the array results. From this data, we are also determining the number of genes that can be analyzed for each set of oocytes collected. For example, we have determined that from 25 oocytes we can make a 1:10 dilution and receive good threshold cycle quantities of approximately 22 to 23 cycles. This indicates that from a starting volume of 20 ul, we can dilute this sample to 100 ul; each RT-PCR requires 5 ul of cDNA starting material, so from a 100 ul starting volume, we could analyze approximately 9 genes in duplicate (including pipetting error).

For these practice analyses, we have successfully designed forward and reverse primers for c-mos and tPA. cDNA is synthesized using SMART technology for reverse transcriptase template switching. Currently we are working on a RT-PCR to determine if the number of oocytes required can be reduced to 5 or 10 using a 1:5 or 1:10 dilution.

### **Experiment 2. Porcine microarray studies.**

Late last fall, we collected mature oocytes from gilt 2-6 mm and sow 2-6 mm follicles for microarray analysis, with 100 oocytes per treatment group. In this trial, we collected only one replicate from each group. Poly A+ RNA was extracted using the OligoTex direct mRNA isolation kit. 5 ul (1/10 RNA) of each sample was removed for G3PDH amplification to confirm the presence of mRNA and run a mock amplification to determine if genomic DNA was present in the samples. The remainder of the samples were dried and submitted to the genomics core facility for two-cycle amplification and hybridization. Both samples produced a G3PDH band after PCR amplification and gel analysis and did not produce a mock band, indicating that the samples did not contain genomic DNA contamination.

A total of 10,766 genes were detected as present or marginal from the 24,123 genes on the porcine specific microarray chip. Of these genes, over 8500 (79%) were in common between the two age groups, demonstrating the similarities between the two age groups during oocyte development. Over 2200 genes (21%) were unique to the sow, suggesting that these represent essential genes acquired with advancing age (Figure 1). Interestingly there were no genes detected that were unique to the gilt oocytes.

From this analysis, we were able to determine the number of genes that were differentially expressed. From the 8533 genes that were in common between mature gilt and so derived oocytes, 7109 genes (83%) displayed similar levels of gene expression. Approximately 1400 genes had greater than a two-fold difference in expression, though over 94% of these genes were increased in the sow oocytes compared to the gilt oocytes. There were 28 and 4 genes that were greater than five-fold and ten-fold different, respectively, in the sow oocytes compared to gilt oocytes. These genes are good candidates of regulatory genes involved in oocyte cytoplasmic maturation that are altered during development. The combined data suggests that there are changes in mRNA levels that are influenced by age further suggesting that gilt oocytes have not completed cytoplasmic maturation and are not competent to undergo normal embryo development.

We began our next step in this experiment in Spring 2006. Mature oocytes were collected from gilt 2-6 mm and sow 2-6 mm follicles for microarray analysis, with 100 oocytes per treatment group. Two replicates from each group were collected. Poly A+ RNA was extracted using the Dynal Bead Kit, which we felt was an improvement over the Oligotex kit. In the oligotex lysis buffer, the SDS in the precipitated out of solution. The dynal kit has LDS which does not precipitate. In this situation we believe that the new lysis buffer would result in increased solubility of oocyte mRNAs. 5 ul (1/10 RNA) of each sample was removed for G3PDH amplification to confirm the presence of mRNA and run a mock amplification to determine if genomic DNA was present in the samples. The remainder of the samples was dried. One replicate from the gilt 2-6 mm group failed to produce a G3PDH band after PCR amplification and gel analysis. The remaining 3 samples did not contain genomic DNA contamination and were submitted to the genomics core facility for two-cycle amplification and hybridization. The remaining gilt 2-6 mm group did not contain enough mRNA to be hybridized onto the microarray. The two sow 2-6 mm groups were returned to Dr. Bidwell, frozen, and the



microarrays were not hybridized. We want to hybridize all the samples from all the treatments simultaneously to avoid variation.

Currently, we have mature oocytes collected from gilt 2-6 mm, sow 2-6 mm, and sow 8-14 mm follicles for microarray analysis, with 150 oocytes per treatment group. Three replicates were collected from both 2-6 mm groups and two replicates were collected from the 8-14 mm group. Mature oocytes were detected using Hepes + 5% sucrose to shrink the oolemma for ease in detecting the extruded polar body. Samples are frozen at -80 in the Krisher lab and are awaiting Poly A+ RNA extraction using the Dynal bead kit. Samples will be extracted and a G3PDH amplification run, followed by hybridization to the Affy arrays.

### **Impact Statement**

- Improvement of in vitro embryo production techniques could improve reproductive efficiency in cattle by accelerating genetic progress on females vs. males. When combined with genetic selection of males using artificial insemination, increases in milk production per cow could increase dairy farm profitability.

*Illinois:*

### **Experiment 1**

Experiment 1 was conducted at the Orr Beef Research Center in Baylis, IL. A total of 252 were included in the experiment. All cows were synchronized with the CO-Synch + CIDR protocol. Cows were inseminated at a predetermined time of 54 hours after the PGF injection and CIDR removal. Cows were then randomly assigned to one of two groups. Cows in the control group received no further treatment. Cows in the CIDR-treated group were administered a once-used CIDR 14 days after the initial timed AI. The CIDR was left in situ for six days and removed 20 days after the initial timed AI. All cows were observed for estrus and inseminated at estrus 18 to 24 days after the initial AI. Pregnancy rates to the initial AI were not different (averaged 40%). Conception rates to the return estrus were 47% and 59% for control and CIDR-treated cows, respectively. Return estrus rates averaged 41%; however, return estrus for the CIDR-treated cows were more ( $P < .05$ ) synchronized—97% of the cows that exhibited estrus were in estrus in a two day period. Cumulative pregnancy rates (1st and 2nd AI) did not differ and averaged 53%.

### **Experiment 2**

Experiment 2 was conducted at the Dixon Spring Agriculture Center in Simpson, Illinois. A total of 471 beef cows suckling calves were included in this study. All cows were synchronized with the CO-Synch + CIDR protocol. Cows were inseminated at a predetermined time of 54 hours after the PGF injection and CIDR removal. Cows were then randomly assigned to one of two groups. Cows in the GnRH-treated group were administered an injection of GnRH (100 mcg) 12 days after the initial AI while cows in the CIDR-treated group were administered a once used CIDR on 14 days after the initial AI. The CIDR was left in situ for six days and removed 20 days after the initial timed AI. All cows were observed for estrus and inseminated at estrus 18 to 24 days after the initial AI. Pregnancy rates to the initial AI were not different (averaged 53%). Conception rates to the return estrus were 66% and 60% for GnRH-treated and CIDR-treated cows, respectively ( $P > .10$ ). Return estrus rates averaged 62%; however, return estrus for the CIDR-treated cows were more ( $P < .01$ ) synchronized. Cumulative pregnancy rates (1st and

2nd AI) did not differ and averaged 72%. Previous studies have demonstrated that the administration of a CIDR through day 21 negatively affected the return estrus conception rate. These data demonstrate that use of a once-used CIDR through day 20 synchronized the return estrus without affecting (or with minimum affect) on the return estrus conception rate. This agrees with data published by Colazo et al. (Theriogenology 65:557-572; 2006) where once-used CIDRs were administered to heifers. In that study conception rates were 64% and 62% for CIDR-treated and control heifers, respectively, and the CIDR improved synchrony of the return estrus.

### **Impact Statement**

- These data suggest that a once-used CIDR may be used for resynchronization without compromising the conception rate. This would permit producers to increase AI calve production by roughly 25% and reduce the number of clean-up bulls needed allowing for increased profits and improved quality of life. Using a conservative published estimate of the greater value of AI calves, resynchronization could increase profits of a 100-cow herd by at least \$3,350.

*Wisconsin:*

### **Experiment 1: Effect of timing of the second GnRH injection of a timed AI protocol on fertility of lactating Holstein cows after first postpartum and Resynch AI services.**

Lactating Holstein cows (n=810) were assigned to one of two Ovsynch protocols with either a 48 or 72 h interval between PGF<sub>2α</sub> (PG) and the second GnRH (G) injection. Cows not previously inseminated received their first postpartum timed AI (TAI) after presynchronization (Presynch) using two doses of PG (25mg) administered 14 days apart, with the second PG injection 14 d before initiation of Ovsynch. Previously inseminated cows received their second or greater TAI (Resynch) using Ovsynch. Approximately half of the cows received Ovsynch using PG and G (100 mg) as follows: (G, d 0; PG, d 7; G+TAI, d 9; Cosynch-48), whereas the remainder received Ovsynch using a 72 h interval between PG and the second G injection as follows: (G, d 0; PG, d 7; G+TAI, d 10; Cosynch-72). Cows were randomized to treatment based on breeding pen, with treatments alternated weekly among four pens. No effect of pen, week, or pen by week interaction was detected. Pregnancy diagnosis was conducted using transrectal palpation at 40.0±0.1 d after TAI. Pregnancy rate per AI (PR/AI) did not differ statistically and was 34% for Presynch and 29% for Resynch, and 29% for Cosynch-48 and 33% for Cosynch-72. There was a tendency (P = 0.11) for Presynch cows receiving Cosynch-72 (n=203) to have a greater PR/AI than cows receiving Cosynch-48 (n=146) (37 vs. 29 %); however, the interval between PG and G during Ovsynch did not affect PR/AI for Resynch cows, (Cosynch-48 = 28%, n=236; Cosynch-72 = 29%, n=225). Sex ratio of calves (n=153) resulting from Cosynch-48 and Cosynch-72 breedings did not differ (48 vs. 43% female, respectively). In summary, Cosynch-72 tended to improve PR/AI for Presynch cows by 8 percentage points, or 22% but did not affect Resynch cows. Thus, extending the interval between PG and the second G injection of Ovsynch by 24 h did not affect PR/AI and may offer more flexibility for implementing a systematic synchronization program.

### **Experiment 2: Assessment of a practical method for identifying anovular dairy cows synchronized for first postpartum timed artificial insemination.**

Lactating Holstein cows (n=842) received a Presynch/Ovsynch protocol using injections of PGF<sub>2α</sub> (PGF; 25 mg) and GnRH (G; 100 μg) to initiate first postpartum (pp) timed AI (TAI) as follows: PGF (39±3 and 53±3 d pp); G (65±3 d pp); PGF (72±3 d pp); G (74±3 d pp) + TAI 16 h later. Two methods for assessing cyclicity status before TAI were compared. For the first method (RIA), blood samples were collected at the 2<sup>nd</sup> PGF of Presynch and at the 1<sup>st</sup> G of Ovsynch, and cows with serum P4 ≥1.0 ng/ml in one or both samples were classified as cycling, whereas cows with serum P4 <1.0 ng/ml in both samples were classified as anovular. For the second method (US), transrectal ultrasonography was used to determine the presence or absence of a corpus luteum (CL) at the 1<sup>st</sup> G of Ovsynch, and cows without CL were classified as anovular, whereas cows with a CL were classified as cycling. Statistical agreement (kappa) between the presence or absence of a CL using US and serum P4 (P4 ≥1.0 ng/ml=CL, P4 <1.0 ng/ml=no CL) at US was 0.74 (P<0.001, good agreement). Sensitivity and specificity of US to determine the presence of a CL was 94.0 and 78.8 %, respectively (53 cows with a CL had serum P4 <1.0 ng/ml). Statistical agreement (kappa) between RIA and US to identify cycling cows was 0.66 (P<0.001, good agreement). Sensitivity, specificity, positive predictive value, and negative predictive value of US to identify cyclicity status was 85.7, 87.7, 64.7, and 95.9 %, respectively. Disagreement between RIA and US to determine cyclicity status occurred because 75 cows had serum P4 ≥1.0 ng/ml at the 2<sup>nd</sup> PGF of Presynch and <1.0 ng/ml at the 1<sup>st</sup> G of Ovsynch. For cows without a CL (n=232), 79.3, 6.9, and 13.8 % had P4 concentrations of <0.5, 0.5 to <1.0, and ≥1.0 ng/ml, respectively. We conclude that assessing the presence or absence of a CL at the first G of Presynch/Ovsynch using US is a practical method for identifying cyclicity status of cows before first TAI but may slightly overestimate the proportion of anovular cows.

### **Experiment 3: Accuracy of pregnancy diagnosis in Holstein cows using transrectal ultrasonography based on a serum pregnancy associated glycoprotein (PAG) ELISA.**

Pregnancy examinations were performed by one herd veterinarian throughout the study using transrectal ultrasonography (US) in lactating Holstein cows (n=877) 27 d after first postpartum timed AI. Outcomes were categorized as: pregnant (PG) = CL, normal uterine fluid, embryo visualized; questionable pregnant 1 (QP1) = CL, normal uterine fluid, embryo not visualized; questionable pregnant 2 (QP2) = CL, abnormal uterine fluid, embryo not visualized; pregnancy loss (PL) = nonviable embryo; nonpregnant (NP) = no CL and/or uterine fluid. Outcomes using US were compared to those categorized PG or NP using a PAG ELISA of plasma samples collected at US. Outcomes for cows in which US and PAG agreed were considered correct, whereas cows in which outcomes disagreed were rechecked using US 32 d after TAI. Outcomes for 112 cows disagreed between US and PAG, and 102 of these cows were rechecked. Statistical agreement (kappa) between PAG and US was 0.74 (P<0.001). Distribution of cows among the US categories was 21.7, 19.7, 4.4, 1.0, and 53.1 % for PG, QP1, QP2, PL and NP categories, respectively. Within each US category, the proportion of cows in which pregnancy outcomes disagreed was 3.7 (7/190), 13.9 (24/173), 64.1 (25/39), 44.4 (4/9), and 11.2 (52/466) % for PG, QP1, QP2, PL and NP categories, respectively. Based on the US recheck, 51.0 and 49.0 % of incorrect outcomes on d 27 were from PAG and US, respectively. Incorrect outcomes for US were 2.1 (4/190), 8.9 (15/168), 52.8 (19/36), 22.2 (2/9) and 2.2 (10/464) % for PG, QP1, QP2, PL, and NP categories, respectively. Overall, PAG incorrectly diagnosed 2.1 (10/467) % of PG cows as NP and 10.5 (42/400) % of NP cows as PG. Thus, although agreement between PAG and US at 27 d after TAI was acceptable, US outcomes of QP1, QP2 and PL (25.2% of all US outcomes) were less accurate than PG or NP outcomes.

#### **Experiment 4: Effects on conception rates of lactating dairy cows by altering the time of the second GnRH and AI during Ovsynch.**

A recent study (Portaluppi & Stevenson, 2005; JDS 88:914) indicated that CoSynch at 72 h after PGF had better conception rates (CR) than Cosynch at 48 h using data from first AI after Presynch. In this study we reexamined these 2 programs at first AI (Presynch) and at later services (Resynch). Further, we hypothesized that CR would be improved to a greater extent when GnRH was administered at 56 h after PGF prior to AI at 72 h due to a more optimal interval (16 h) between the LH surge and AI. A total of 1507 AIs in 927 lactating dairy cows were randomly assigned to one of three treatments by pen from August to December 2005. Cows ranged from 30 to 36 DIM at start of Presynch (two injections of PGF 14 d apart with the second injection 11 d before Ovsynch). All cows received GnRH followed 7 d later by PGF and then received one of the following: 1) GnRH + TAI 48 h after PGF (G48) 2) GnRH 56 h after PGF + TAI 72 h after PGF (G56) or 3) GnRH + TAI 72 h after PGF (G72). Pregnancy diagnoses were performed by ultrasound at 31 to 33 d post-AI and again at 52-54 d post-AI. CR were similar ( $P>0.1$ ) for the G48 and G72 groups (26.7% and 27.2%, respectively). The G56 group had a much greater CR (36.2%) than G48 ( $P=0.006$ ) or G72 ( $P=0.002$ ) groups. Cows at first AI (Presynch) had greater CR than cows at later AIs (Resynch) in G48 (37.8 vs. 23.6%;  $P=0.002$ ) and G56 (45.2 vs. 33.1%;  $P=0.021$ ) but not in G72 (27.5 vs. 27.3%;  $P>0.1$ ). Similarly, primiparous cows had greater CR than multiparous cows in G48 (34.1 vs. 23.1%;  $P=0.009$ ) and G56 (41.3 vs. 32.7%;  $P=0.065$ ) but not G72 (29.8 vs. 25.4%;  $P=0.264$ ). Pregnancy loss was higher for primiparous than multiparous cows in the G48 and G56 groups but not in G72 ( $P=0.02$ ,  $P=0.016$ , and  $P>0.1$ , respectively). In conclusion, we found no advantage to Cosynch at 72 h vs. 48 h either at first or later AIs. In contrast, we found a clear advantage to treating with GnRH at 56 h prior to a 72 h AI probably due to the more optimal timing of AI prior to ovulation.

#### **Impact Statement**

- Results from current and ongoing research have been used to make recommendations to over 1,200 stakeholders and their consultants in Wisconsin and throughout the U.S. regarding implementation of systematic synchronization and resynchronization systems for lactating dairy cows as well as timing and methods for pregnancy diagnosis. Data generated in these projects has been published in scientific journals and included in numerous extension proceedings.

*Iowa:*

#### **Experiment 1. Influence of environmental agents on reproductive potential**

Phytoestrogens, such as genistein, can bind to both the  $\alpha$  and  $\beta$  estrogen receptor and act as partial agonists and/or antagonists. Because grazing cattle can be exposed to various phytoestrogens, we are investigating their impact on ovarian function. Ovaries were collected from cycling heifers during the first wave of follicular development. The dominant and two largest subordinate follicles of the first wave of follicular development were collected, and pieces of follicle wall cultured for 24 hours in the presence of FSH with or without genistein. Estradiol was measured in the conditioned media by RIA, and levels of aromatase determined in the

tissues after culture by RT-PCR. Overall, the dominant follicle secreted more estradiol in culture than either of the two subordinate follicles ( $p < 0.05$ ). Although there was no significant effect of genistein on the ability of follicular tissue to secrete estradiol in culture, this phytoestrogen did decrease levels of aromatase in tissue collected from the dominant follicle and treated with FSH, compared to levels with FSH alone ( $p < 0.05$ ). These findings indicate that genistein may alter the ability of follicular tissue to produce estradiol and that this may impact circulating concentrations not acutely, but over the course of the estrous cycle.

### **Experiment 2. Expression of Peroxisome Proliferator-Activated Receptor in the Bovine Ovary**

PPAR $\gamma$  is a member of the steroid receptor superfamily. We have shown that in the rat, relative expression of PPAR $\gamma$  mRNA is low in newly forming CL and higher in CL from past cycles, PPAR $\gamma$  expression is inversely related to the expression of P450 side-chain cleavage, and PPAR $\gamma$  does not play a major role in luteal progesterone production. This latter finding differs work by Lohrke *et al.* (J. Endo 1998 159:429) which reported that PPAR $\gamma$  stimulated progesterone production from mid-cycle bovine luteal cells. The following study was conducted to investigate if the disparate role of PPAR $\gamma$  in progesterone production between rats and cattle was due to differences in luteal formation/function. CL was collected from cycling heifers on the following days post-ovulation ( $n=3$ ): 4; 12; 12, 24 hours post-treatment with PGF $2\alpha$  to initiate luteal regression; and 17. PPAR $\gamma$  mRNA and protein were measured by RT-PCR and western immunoblot analysis, respectively. Pieces of CL were also cultured alone or with LH (100 ng/ml), agonists of PPAR $\gamma$  (PGJ2: 5 and 65  $\mu$ M; ciglitazone: 5 and 25  $\mu$ M) or a PPAR $\gamma$  antagonist (GW9662: 0.1 and 1.0  $\mu$ g/ml). After 24 hours media were collected and progesterone was measured by RIA. Levels of mRNA for PPAR $\gamma$  were relatively steady throughout the luteal phase, however there was a trend towards lower expression in CL collected on day 17 compared to day 12 ( $p=0.06$ ). The amount of PPAR $\gamma$  protein in CL on days 4 and 12 was relatively high, and reduced in CL collected post-PGF $2\alpha$  and on day 17 compared with mid-cycle CL ( $p < 0.05$ ). Treatment with the PPAR $\gamma$  agonist, ciglitazone (25  $\mu$ M), significantly decreased secretion of progesterone by CL collected on day 17 post-ovulation. These data indicate that the expression of PPAR $\gamma$  in bovine luteal tissue differs from that in the rat with relatively high expression in newly forming CL, and a decrease in the protein in regressing CL. Ciglitazone did not stimulate progesterone secretion from mid-cycle CL which differs from the findings of Lohrke *et al.* and may be due to the fact that in the current study pieces of bovine CL were cultured, whereas the earlier study used dispersed luteal cells. The relatively high expression of PPAR $\gamma$  in newly forming and mid-cycle CL, and the ability of agonists of PPAR $\gamma$  to affect progesterone secretion suggest that in cattle, PPAR $\gamma$  may play a role in luteal formation and/or function.

### **Experiment 3. Immunocytochemical distribution of mammotrophs in porcine anterior pituitary (J. Lee, M.H. Stromer, S. Jeftinija, K. Jeftinija, C.G. Scanes, L.L. Anderson)**

Prolactin (PRL) is synthesized in specific anterior pituitary secretory cells, mammotrophs or PRL cells (lactotrophs) and has diverse functions including growth, reproduction, lactation, osmoregulation, immunomodulation, and energy metabolism. The objective of our study was to identify spatial distribution patterns of mammotrophs in the newborn and prepubertal porcine pituitary using fluorescence immunocytochemistry. Immunoreactive mammotrophs were polygonal or irregular shape with unstained nuclei and ranged from 10 to 15  $\mu$ m in diameter in 1-, 45-, and 90-day old pigs. Clusters of mammotrophs consisting of 3 to 10 cells were found in

day 45 and day 90 old pigs. Significant differences were observed among the total mammotrophs per  $30,495 \mu\text{m}^2$  across the three age groups (day 1; day 45 and day 1; day 90,  $P < 0.0001$ ). Anterior lobes were sampled at 3 positions (a, b, and c) in each of 5 radial regions (1-5) in each of 3 levels (proximal, middle, and distal) perpendicular to the gland axis. There were changes in spatial distribution of mammotrophs with different levels of the gland and this specific pattern is similar among different age groups. Mammothrophs were most numerous in positions a and b ( $57 \pm 4.2$ ; mean  $\pm$  standard error of the mean per  $30,495 \mu\text{m}^2$ ), and least numerous in position c at the proximal level ( $24 \pm 5.2$ ) in day 1, day 45, and day 90 indicating high population and immunointensity close to intermediate lobe. However, at the distal level, the pattern showed a bell shape with significant increase in the number of mammothrophs in region 3 at position c ( $P < 0.001$ ). From these results, we suggest that there may be regional specificity of cellular differentiation and transformation to control PRL secretion to meet the need for endocrine regulation as the animal ages.

#### **Experiment 4. Immunocytochemical distribution of mammosomatotrophs in porcine anterior pituitary** (J. Lee, M.H. Stromer, S. Jeftinija, K. Jeftinija, C.G. Scanes, L.L. Anderson)

The existence of a novel anterior pituitary cell type, the mammosomatotroph (MS), secreting both growth hormone (GH) and prolactin (PRL) was proposed to function as transitional cells or progenitor cells between GH cells (somatotrophs) and PRL cells (mammotrophs). Double fluorescence immunocytochemistry was used to identify distribution patterns of MS cells in anterior pituitary glands from newborn and prepubertal stages of pigs. Immunopositive MS cells were morphologically similar to the mammothrophs including polygonal or irregular shape and ranged from 10 to 15  $\mu\text{m}$  in diameter. The confocal detection of a MS demonstrated uneven distribution of GH and PRL. Anterior lobes were sampled at 3 positions in each of 5 radial regions in each of 3 levels perpendicular to the gland axis. There were changes in spatial distribution with different levels of the gland and this specific pattern is similar among different age groups (day 1, day 45, and day 90). Although MS is rare in number, significant differences were observed among the total MS cells across three age groups ( $P < 0.0001$ ). MS cells were most numerous in region 3 where originates from embryonic Rathke's pouch ( $6.0 \pm 1.4$ ; mean  $\pm$  standard error of the mean per  $75,264 \mu\text{m}^2$ ) compare to rest of regions ( $0.7 \pm 0.1$ ). There were significant decreases at position a ( $P < 0.001$ ) and significant increases ( $P < 0.0001$ ) at position c in region 3 from proximal to distal level. The total number of MS cells at the proximal level at position an increased from day 1 to day 45 (2.5 $\times$ ) and from day 45 to day 90 (2.4 $\times$ ). The results suggest that there may be regional specificity of cellular transformation to facilitate GH and PRL secretion during the rapid growing period in the young pig.

#### **Impact Statements**

- PPAR has been detected in ovarian tissue from cattle and activation of PPAR in late stage luteal tissue inhibited progesterone production. PPAR $\gamma$  is activated by factors in an animal's diet (fatty acids) and environment (phytoestrogens). Therefore, this transcription factor may mediate the influence of nutrition on ovarian gene expression, affecting female fertility.
- Genistein, a phytoestrogen found in various cattle feedstuffs, can alter the expression of enzymes involved in steroidogenesis. This may be one mechanism by which feeds high in phytoestrogens cause reproductive problems such as irregular estrus, cystic ovaries, and decreased conception rates.

- There may be regional specificity within the pituitary regarding the differentiation and transformation of mammotrophs as the animal ages to control PRL and growth hormone secretion. These findings indicate coordinated development of somatomammotrophs with animal growth. Interruption of this relationship may delay growth and decrease production efficiency.

*Missouri:*

### **Experiment 1**

The key period of risk for disease (metabolic disorders, metritis and mastitis) is during the periparturient period. High producing dairy cows are in a negative energy balance during the transition from late gestation to early lactation. Higher serum levels of (NEFA) and lower serum levels of glucose indicate a negative energy balance in postpartum dairy cows. The overall objective was to examine the relationship between metabolic indicators, body condition, changes in body condition and the animal's health during lactation, ovarian cyclicity, conception rates and circulating hormone concentrations. The general hypothesis was that the metabolic indicators and animal health will be related to differences in energy balance during the periparturient period through early lactation which will be related to changes in body condition and conception rates and will alter hormone concentrations. Preliminary data are presented to show a negative relationship between conception rate and blood NEFA levels.

A preliminary study was conducted to determine the relationship between NEFA and glucose levels in periparturient dairy cows (n=89) and subsequent fertility (i.e. pregnancy). The cows used in the study were cows that were included in the MO station contribution of the NC-1006 dairy project. Briefly, approximately 40 d postpartum, cows began one of two timed artificial insemination protocols that included Presynch treatments (PGF<sub>2α</sub>, 14d later PGF<sub>2α</sub>, 14d later GnRH, 7d later PGF<sub>2α</sub>, 2d later GnRH). Cows were randomly assigned by lactation to be bred either at the last GnRH (CoSynch) or 24h later (OvSynch). Pregnancy was determined at 32d post-breeding and then again at 60d after breeding by ultrasonography. In addition, serum samples were taken at approximately 10, 7, and 3d prepartum and 3, 7, 14, and 21d postpartum and concentrations of non-esterified fatty acids (NEFAs) and glucose were measured. Comparisons were made between cows that became pregnant vs. those that were diagnosed open at the timed insemination. Data were analyzed using a mixed model analysis of variance for repeated measures. Pregnancy rates for d30 and d60 are shown in Table 1. Across all days, NEFA levels were lower (p <0.0001) and glucose levels were higher (p <0.0001) for cows that subsequently became pregnant at first service versus those that remained open (Fig.1 and 2). Means (±SE; μmol/L) of pregnant and open cows at 3d postpartum were 491±70 and 746±49 of NEFA and were 56.4±1.5 and 51.6±1.0 of glucose, respectively.

### **Impact Statement**

- Completion of the cooperative proposal will define optimum breeding time relative to GnRH injection and the ability to identify anestrus during the postpartum period with heat detection devices.

*South Dakota:*

### **Experiment 1. Effect of heifer nutrition following artificial insemination on pregnancy success.**

Since nutrition can play a vital in the success of any artificial insemination program. The question has often been asked if moving heifers from the feedlot to pasture following a synchronized AI program effects pregnancy success. This study was designed to determine if moving heifers from a feedlot to pasture immediately following insemination will influence pregnancy success.

Three hundred thirty-three pasture developed Angus beef heifers at one location were synchronized with the Select Synch-CIDR protocol and AI was performed following detection in standing estrus by a single inseminator. At insemination heifers were randomly assigned to one of three treatments: 1) heifers remained in the feedlot on the same ration, 2) heifers were immediately moved to pasture, or 3) heifers were immediately moved to pasture and supplemented with 5 lbs•hd-1•day-1. Blood samples were collected on d 0 (d of CIDR insertion), AI, 11, 21 (clean up bulls turned out), and 49 (pregnancy determination). Body condition scores were determined at AI and at pregnancy determination).

Body condition score did not differ ( $P = 0.78$ ) among treatments at the initiation ( $5.41 \pm 0.05$ ,  $5.40 \pm 0.05$ ,  $5.37 \pm 0.05$  for feedlot, moved to pasture, and moved to pasture and supplemented, respectively). Synchronized pregnancy rates were similar ( $P > 0.64$ ) among heifers left in the feedlot, moved to pasture, and moved to pasture and supplemented (56%, 59%, and 57%, respectively). At pregnancy determination BCS and change in BCS during the treatment period did differ ( $P < 0.0001$ ) among treatments. At the end of treatment heifers on pasture and supplemented had the greatest ( $P < 0.01$ ) change in condition (BCS  $5.94 \pm 0.04$ ; change  $0.57 \pm 0.06$ ) compared to heifers left in the feedlot (BCS  $5.79 \pm 0.04$ ; change  $0.38 \pm 0.06$ ) or heifers moved to pasture (BCS  $5.42 \pm 0.05$ ; change  $0.02 \pm 0.06$ ). Final pregnancy rates did not differ ( $P > 0.40$ ) among treatments (85%, 89%, and 88% for heifers left in the feedlot, moved to pasture, and moved to pasture and supplemented, respectively). In summary when heifers are developed on forage moving them from a feedlot to a pasture with or without supplementation had no influence on pregnancy success.

### **Experiment 2**

Non-lactating beef cows ( $n = 19$ ) were treated with the CO-Synch protocol (100 $\mu$ g GnRH on d -9; 25 mg PG on d -2; and 100 $\mu$ g GnRH on d 0). Half ( $n = 10$ ) the cows received an injection of estradiol cypionate (ECP; 1mg) 12 h following PG. Cows detected in standing estrus within 24 h of the second GnRH injection were considered to be in standing estrus. Uterine pH was determined at time of ECP administration and daily through ovulation.

Cows treated with ECP had greater ( $P < 0.01$ ) concentrations of estradiol compared to non-treated cows ( $8.3 \pm 0.7$  and  $5.2 \pm 0.7$  pg/mL, respectively). A treatment by time interaction ( $P < 0.01$ ) influenced concentrations of estradiol. All cows had similar ( $P > 0.15$ ) concentrations of estradiol at time of ECP, but ECP treated cows had elevated ( $P < 0.02$ ) concentrations of estradiol following the second GnRH injection compared to control cows. Treatment ( $P = 0.01$ ), time ( $P < 0.01$ ), and treatment by estrus by time ( $P = 0.065$ ) influenced uterine pH. Control cows that did not exhibit estrus had a higher uterine pH compared to ECP cows that did not exhibit estrus ( $P = 0.03$ ) at time of ECP. All cows had a similar uterine pH ( $P > 0.19$ ) 12 h after ECP. Control cows that did not exhibit estrus had a higher uterine pH compared to control cows that did exhibit estrus ( $P < 0.01$ ) and ECP cows that exhibited estrus ( $P = 0.05$ ) at time of the second GnRH injection (time insemination would occur;  $7.0 \pm 0.1$ ,  $6.7 \pm 0.1$ ,  $6.8 \pm 0.1$ ,



respectively). ECP cows not exhibiting estrus were intermediate ( $6.8 \pm 0.1$ ). All cows had similar uterine pH 24 h after GnRH through ovulation ( $P > 0.06$ ). Concentrations of estradiol had no linear ( $P > 0.21$ ) or quadratic ( $P > 0.21$ ) relationship with uterine pH. In summary, ECP treatment elevated concentrations of estradiol and lowered uterine pH to a level similar to the uterine pH of control cows that exhibited estrus within 24 h of when insemination would occur.

### Experiment 3

Lactating postpartum beef cows ( $n = 40$ ) were treated with the CO-Synch protocol (100 $\mu$ g GnRH on d -9; 25 mg PG on d -2; and 100 $\mu$ g GnRH on d 0). Half ( $n = 20$ ) the cows received an injection of estradiol cypionate (ECP; 1mg) 12 h following PG. Cows detected in standing estrus within 24 h of the second GnRH injection were considered to be in standing estrus. Uterine pH was determined at time of ECP administration and at 12 hour intervals until the second GnRH injection; following the second GnRH injection uterine pH was determined at 6 hour intervals until ovulation.

All animals administered ECP exhibited standing estrus within 24 h of the second GnRH injection. Control cows that did not exhibit estrus had a higher uterine pH compared to ECP cows ( $P = 0.002$ ) and control cows that exhibited standing estrus ( $P = 0.01$ ) at time of the second GnRH injection. All cows had a similar uterine pH ( $P > 0.19$ ) 6 h after ECP.

### Impact Statement

- Insemination of cows that have not seen a preovulatory rise in estradiol may have decreased pregnancy rates as a result of improper uterine environment at time of insemination.

*USDA MARC:*

### Impact Statement

- Total pounds of calf weaned influences profitability for the cow-calf producer. We have validated a polymorphism in the Osteopontin gene as a genetic marker for birth weight and weaning weight in the USMARC Twinning population and examined its association with reproductive phenotypes (ovulation rate and twinning). Results show that the favorable allele increases birth weight and weaning weight in singletons without negatively affecting reproduction and could increase calf survival. In offspring homozygous for the positive allele, increasing weaning weight by 23 lbs could increase profits per cow substantially.

*Kentucky:*

**Heifer Experiments:** Two methods for synchronization of estrus in dairy heifers are currently being compared. Holstein, jersey and crossbred heifers, at least 14 months of age, are randomly assigned to either method. Method 1 is a traditional prostaglandin F2a (PGF) based protocol consisting of 2 injections of PGF (25 mg; Lutalyse, Pfizer Animal Health) administered 14 days apart. An injection of estradiol cypionate (.25 ug; ECP, Pfizer Animal Health) is then administered 24 h after the second injection of PGF. Method 2 is a CIDR based protocol. CIDRs (Pfizer Animal Health) are inserted then removed after 14 days. PGF (25 mg) is administered 18 days after CIDR removal. An injection of estradiol cypionate (.25 ug; ECP, Pfizer Animal Health) is then administered 24 h after injection of PGF. Estrus detection (30 min every 12 h) is initiated immediately after the ECP injections. Heifers are bred 12 h after first

detection of estrus. Endpoints for the experiment are expression of estrus and conception at breeding. To date, 58 heifers have completed the PGF based protocol and 63 heifers have completed the CIDR based protocol. The experiment will be complete when at least 80 heifers have completed each protocol.

**Experiment Title: Effect of Physiological State on Concentrations of Progesterone**

**Maintained by CIDRs.** The concentration of progesterone achieved by CIDRs is being compared in nonpregnant Holstein cattle during three physiological states: lactation, after drying off and in heifers (12-15 months of age). In experiment 1, six animals in each physiological state were examined ultrasonographically to confirm the presence of a corpus luteum. If present, a coccygeal venous blood sample was collected and a CIDR was inserted. The day of CIDR insertion was designated day 0. CIDRs were removed at 0700 on day 7. Coccygeal blood samples were collected at 12-h intervals through 1900 on day 9 (samples were collected at 0700 and 1900 h). Prostaglandin F<sub>2a</sub> (25 mg) was administered immediately after sample collection at 0700 on day 1 and again at 1900 on day 1 to induce luteolysis. Luteolysis was complete by 1900 on day 2. The mean concentration of progesterone maintained in each cow from 0700 on day 3 through 0700 on day 7 was calculated as a measure of the concentration of progesterone maintained by the CIDR. Body weight was recorded at 0700 on day 0 and at 0700 on day 9. Milk production was recorded at each milking for the lactating cows. **Results:** Concentrations of progesterone were high at the onset of the experimental period since all of the animals were in the luteal phase. PGF<sub>2α</sub> (black arrows) induced luteolysis; however, concentrations of progesterone were maintained above basal levels by the CIDRs. Immediately following removal of the CIDRs, concentrations fell to approximately 0.05 ng/ml. During the post luteolytic period (0700 on day 3 through 0700 on day 7), the concentration of progesterone maintained by the CIDR was affected by the physiological state of the animals. Concentrations were greatest in heifers, least in lactating cows and intermediate in dry cows.

**Impact Statement:**

- Improved procedures for estrous synchronization in heifers and lactating dairy cows will increase farm income by increasing milk production, through reduced days open and genetic improvement (widespread use of artificial insemination). The increase in milk production is conservatively valued at \$100/cow.

*Nebraska:*

*Heifer development-effects of soybeans or DDG on intrafollicular VEGF expression*

Vascular endothelial growth factor (VEGF) is involved in bovine follicle maturation, ovulation and corpus luteum development. Alternative splicing of the VEGF gene produces both angiogenic and inhibitory isoforms of the protein. The predominant angiogenic isoforms are VEGF<sub>164</sub> and VEGF<sub>120</sub>. The objective of this study was to determine whether heifer nutrition influences VEGF expression in dominant follicles. Crossbred yearling beef heifers were assigned randomly to receive 1.65 kg soybeans ground with 0.40 kg corn (SOY; n = 12) or an isonitrogenous, isocaloric control diet (DDG; n = 10) for 157 days during the development phase. The SOY diet contained more lipid and three phytoestrogens were detected in the soybeans. Heifers were synchronized with two shots of prostaglandin F<sub>2α</sub> (PGF) administered 14 days apart, and dominant follicles were measured and aspirated 60 hours following the second

PGF injection using transvaginal ultrasonography. Quantitative RT-PCR was conducted on granulosa cell aspirates to quantify VEGF isoform expression relative to GAPDH expression. Only samples from follicles with an estrogen to progesterone ratio > 1 in the follicular fluid were included in the analysis. Dominant follicle diameter was greater ( $P = 0.09$ ) for SOY than DDG heifers at the time of aspiration ( $12.23 \pm 0.43$  mm vs.  $11.10 \pm 0.47$ , respectively). However, follicular fluid estrogen and progesterone concentrations, and the ratio of estrogen to progesterone, were similar. Expression of VEGF120 was greater ( $P = 0.05$ ;  $4.74 \pm 0.96$  vs  $2.04 \pm 0.82$ ) in granulosa cell extracts from DDG heifers than SOY heifers. Additionally, expression of VEGF164b was greater ( $P = 0.05$ ) in granulosa cells from DDG heifers than SOY heifers, but expression of VEGF164 did not differ between groups ( $P = 0.36$ ). Simple correlation analysis failed to demonstrate a correlation between VEGF120 or VEGF164b expression and follicle diameter, follicular fluid estrogen concentration, or follicular fluid progesterone concentration, suggesting that VEGF120 and VEGF164b expression are altered independently of these parameters. These data demonstrate that nutrition can modulate VEGF120 and VEGF164b but not VEGF164 expression in bovine dominant follicles.

#### *Heifer development – effects of feeding DDG as an energy source*

A 2-yr study was conducted to determine if supplementing beef heifers with dried distillers grains (DDG) as an energy source affected age at puberty or reproductive performance. Spring-born crossbred heifers were blocked by sire and age (location one,  $n = 165$ ) or age (location two,  $n = 151$ ) and assigned randomly to DDG or CON treatments. The control supplement contained dried corn gluten feed, whole corn germ, and urea. Heifers received ad libitum prairie hay (11% CP, 54% TDN, location one; 8.4% CP, 53.9% TDN, location two), and either 0.57% of BW DDG or 0.73% of BW CON (DM basis). Diets were formulated to be isonitrogenous and isocaloric, however protein degradability differed. Supplemental UIP intake from DDG averaged  $253 \text{ g}\cdot\text{hd}^{-1}\cdot\text{d}^{-1}$  and reached  $301 \text{ g}\cdot\text{hd}^{-1}\cdot\text{d}^{-1}$ , CON supplemental UIP intake averaged  $92 \text{ g}\cdot\text{hd}^{-1}\cdot\text{d}^{-1}$  and peaked at  $111 \text{ g}\cdot\text{hd}^{-1}\cdot\text{d}^{-1}$ . Supplementation began 38 d after weaning for an average of 194 d. Initial pubertal status was determined by two blood samples collected 10 d apart. Weights and blood samples were collected monthly for the first 3 mo and at 14 d intervals thereafter, with supplement intake adjusted accordingly. Heifers were synchronized with two injections of prostaglandin  $F_{2\alpha}$  14 d apart, heat detected for 5 d, inseminated 12 h after detection of estrus, and placed with bulls for approximately 50 d beginning 9 d after the final AI. Conception and pregnancy rates were determined via transrectal ultrasonography.

There was no difference between groups in age, initial weight, initial BCS, or final BCS. Furthermore, weight and BCS at final pregnancy determination were not influenced by supplementation. There was a treatment by location interaction for final weight and ADG. Final weights and ADG were similar between groups at location one but were greater ( $P < 0.05$ ) for DDG heifers than CON heifers at location two.

Supplement type did not influence the proportion of heifers that had achieved puberty prior to synchronization, or the average age at puberty. Weight at puberty was greater ( $P = 0.03$ ) for DDG heifers than CON heifers, primarily due to the higher ADG and final weight of DDG heifers at location two. A similar percentage of heifers from CON and DDG were detected in estrus within 5 d following the final PGF injection, and the timing of observed estrus was similar between groups. Conception rate to AI was greater ( $P = 0.0004$ ) for DDG than CON heifers (52.9% vs. 75.0%). Furthermore, AI pregnancy rates were greater ( $P = 0.003$ ) for DDG heifers

than control heifers (40.1% vs. 57.0%). Overall pregnancy rates following exposure to bulls were similar between DDG and CON heifers (91.8% vs. 87.3%).

*Heifer development – effect of dam nutrition on subsequent reproductive performance*

A 3-yr study was conducted with heifers (n = 170) whose dams were used in a 2x2 factorial to determine effects of late gestation or early lactation dam nutrition on subsequent heifer growth and reproductive performance. During the last trimester of gestation, cows received either 0.45 kg/d of 42% CP supplement or no supplement while grazing dormant winter range. During early lactation, after the end of the calving season and immediately prior to the breeding season, cows were either fed cool-season grass hay or grazed sub-irrigated meadow. Cows were managed in a common group the remainder of the year. Heifer birth date and birth weight were not affected by late gestation or early lactation dam nutrition. Spring meadow grazing and PS increased ( $P = 0.02$ ;  $P = 0.07$ ) heifer 205-d weight. Pre-breeding weight and weight at pregnancy determination were greater ( $P = 0.04$ ;  $P = 0.03$ ) for heifers from PS dams, but early lactation nutrition did not affect either weight. There was no effect of late gestation or early lactation dam nutrition on age at puberty or percentage of heifers cyclic before breeding, and no difference in pregnancy rates or calving data due to early lactation nutrition of the dam. However, overall pregnancy rates were greater (93% vs. 80%,  $P = 0.05$ ) for heifers from PS dams, and a greater proportion of heifers born to PS dams calved in the first 21 d of the calving season as 2-yr. olds (77 % vs. 49%,  $P = 0.003$ ). Heifers born to PS cows calved earlier ( $P = 0.07$ ) in their initial calving season, had a greater proportion unassisted births ( $P = 0.03$ ), but similar ( $P = 0.61$ ) calf birth weights. Weight at the beginning of the second breeding season was greater ( $P = 0.005$ ) for heifers from PS dams than NS dams but was not affected by maternal nutrition during early lactation. Dam nutrition did not affect individually-fed heifer ADG or G:F ratio. There was an late gestation x early lactation interaction for DMI ( $P = 0.09$ ) and residual feed intake (RFI;  $P = 0.07$ ). Heifers from PS dams had greater DMI ( $P = 0.09$ ) and RFI ( $P = 0.07$ ) if their dams were fed hay during early lactation, but not if their dams grazed meadows.

A subsequent project is in progress comparing the effects of third trimester protein supplementation of cows on winter range and corn stalks on heifer growth and reproductive performance.

## **Future Work:**

## **Collaborative Studies:**

Contributing stations plan to continue their work on experiments designed to address Objectives 1 and 2.

## **Publications:**

### *Minnesota:*

#### **Journal Articles**

- Lamb, G.C., J.E. Larson, T.W. Geary, J.S. Stevenson, S.K. Johnson, M.L. Day, R. P. Ansotegui, D. J. Kesler, J.M. DeJarnette, and D. Landblom. 2004. Synchronization of estrus and artificial insemination in replacement beef heifers using GnRH, PGF<sub>2</sub>α and progesterone. *J. Anim. Sci.* (In press).
- Santos, N.R., G.C. Lamb, H.B. Roman, R.O. Gilbert. Postpartum endometrial cytology in beef cows. *Theriogenology* (In press).
- Larson, J. E., G. C. Lamb, J. S. Stevenson, S. K. Johnson, M. L. Day, T. W. Geary, D. J. Kesler, J. M. DeJarnette, F. N. Schrick, A. DiCostanzo, and J. D. Arseneau. 2006. Synchronization of estrus in suckled beef cows for detected estrus and artificial insemination and timed artificial insemination using gonadotropin-releasing hormone, prostaglandin F<sub>2</sub>α, and progesterone. *J. Anim. Sci.* 84:332-342.

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- Lamb, G.C. 2005. Donor and recipient factors affecting an embryo transfer program. 2005 American Embryo Transfer Association and Canadian Embryo Transfer Association Joint Convention Proceedings 76-83.
- Lamb, G.C., J.E. Larson, G. Marquenzini, J Vasconcelos. 2005. Factors Affecting Pregnancy Rates in an IVF Embryo Transfer Program. 2005 American Embryo Transfer Association and Canadian Embryo Transfer Association Joint Convention Proceedings 31-36.
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- Lamb, G.C. and J.E. Larson. 2005. Review of Estrous Synchronization Systems - CIDR. 5th Applied Reproductive Strategies in Beef Cattle, College Station, TX pp 95-105.
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- Lamb, G.C. 2005. Factors affecting and embryo transfer program. 4th Applied Reproductive Strategies in Beef Cattle, Lexington, KY pp 257-267.
- Lamb, G.C. 2005. Ultrasound – early pregnancy diagnosis and fetal sexing. 3rd Applied Reproductive Strategies in Beef Cattle, Reno, NV pp 253-262.
- Lamb, G.C. 2005. Ultrasound – early pregnancy diagnosis and fetal sexing. 4th Applied Reproductive Strategies in Beef Cattle, Reno, NV pp 268-278.

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- Santos, N.R., G. C Lamb, H. B. Roman, R. O. Gilbert. 2005. Postpartum endometrial cytology in beef cows. Society for Theriogenology (Abstract).
- Carriquiry, M., W. J. Weber, C. R. Dahlen, G. C. Lamb, and B. A. Crooker. 2005. Effects of bST and dietary fat in early lactation on lactational performance of Holstein cows. J. Anim. Sci. 83 (Suppl. 1):179.
- Dilorenzo, N., C.R. Dahlen, A. DiCostanzo, and G.C. Lamb. Feedlot performance response by steers to oral doses of polyclonal antibody preparations against *Streptococcus bovis* or *Fusobacterium necrophorum*. J. Anim. Sci 83 (Suppl. 1):369.
- Thielen, K. N., J. E. Larson, B. J. Lovaas, D. J. Kesler, J. S. Stevenson, T. T. Martson and G. C. Lamb. 2006. Influence of CIDR insert after a fixed-time AI on pregnancy rates and return to estrus of nonpregnant cows. J. Anim. Sci. 84 (Suppl. 1):432.
- Lamb, G. C., C. R. Dahlen, K. A. Vonnahme, G. R. Hansen, J. D. Arseneau, G. A. Perry, J. Clement, and J. D. Arthington. 2006. Effects of estrous synchronization with a CIDR prior to the breeding season in bull-breeding herds on pregnancy rates. J. Anim. Sci. 84 (Suppl. 1):433.
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- Carriquiry, M., C. R. Dahlen, W. J. Weber, G. C. Lamb, and B. A. Crooker. 2006. Bovine somatotropin and dietary fat enriched with omega-3 fatty acids in dairy cows: III. Postpartum ovarian activity. J. Anim. Sci. 84 (Suppl. 1):346.

*Kansas:*

### **Journal Articles**

- Blevins, C. A., J. E. Shirley, and J. S. Stevenson. Milking frequency, estradiol cypionate, and somatotropin influence lactation and reproduction in dairy cows. J. Dairy Sci. 89: In press.
- Stevenson, J. S., M. A. Portaluppi, D. E. Tenhouse, A. Lloyd, D. R. Eborn, S. Kacuba, and J. M. DeJarnette. Post-AI Interventions: Conception rates, pregnancy survival, and ovarian responses to GnRH, hCG, and progesterone. J. Dairy Sci. 89: In press.
- Stevenson, J.S., J.R. Pursley, H.A. Garverick, P.M. Fricke, D.J. Kesler, J.S. Ottobre, and M.C. Wiltbank. 2006. Treatment of cycling and noncycling lactating dairy cows with progesterone during Ovsynch. J. Dairy Sci. 89:2567-2578.
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- Stevenson, J. S., M.A. Portaluppi, and D.E. Tenhouse. 2006. Delaying injection of prostaglandin F<sub>2</sub>alpha in an Ovsynch protocol. *J. Dairy. Sci.* 89(Suppl. 1):149 (Abstr.).
- Stevenson, J. S., D.E. Tenhouse, M.A. Portaluppi, and A. Lloyd. 2006. Post-AI interventions in lactating dairy cattle. I. Ovarian responses to GnRH, hCG, and exogenous progesterone (CIDR). *J. Dairy. Sci.* 89(Suppl. 1):47 (Abstr.).
- Stevenson, J. S., D.E. Tenhouse, M.A. Portaluppi, D.R.Eborn, S. Kacuba, and J.M. DeJarnette. 2006. Post-AI interventions in lactating dairy cattle II. Conception rates and pregnancy survival in response to GnRH, hCG, and exogenous progesterone (CIDR). *J. Dairy. Sci.* 89(Suppl. 1):48 (Abstr.).
- Portaluppi, M.A., J.S. Stevenson, and D.E. Tenhouse. 2006. Timed AI conception rates after variations of the Ovsynch protocol in dairy cattle. *J. Dairy. Sci.* 89(Suppl. 1):435 (Abstr.).
- Dobbins, C.A., D.E. Tenhouse, D.R.Eborn, K.R. Harmony, S.K. Johnson, and J.S. Stevenson,. 2006. Conception rates after altered timing of AI associated with the CO-synch + CIDR protocol. *J. Anim. Sci.* 84(Suppl. 1):50 (Abstr.).
- Thielen, K.N., J.E. Larson, B.J. Lovaas, D.J. Kesler, J.S. Stevenson, T.T. Marston, and G.C. Lamb. 2006. Influence of a CIDR insert after a fixed-time AI on pregnancy rates and return to estrus of nonpregnant cows. *J. Anim. Sci.* 84(Suppl. 1):432 (Abstr.).

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- Herrick, J.R., Behboodi, E., Memili, E., Blash, S., Echelard, Y., Krisher, R.L. (2006) Metabolism, protein content and in vitro embryonic development of goat cumulus-oocyte complexes matured with physiological concentrations of glucose and L-lactate. *Mol. Reprod. Dev.* 73:256-266. Published Online: 25 Oct 2005.
- Herrick, J.R., Brad, A.M., Krisher, R.L. (2006) Chemical Manipulation of Glucose Metabolism in Porcine Oocytes: Effects on Nuclear and Cytoplasmic Maturation In Vitro. *Reprod.* 131: 289-298.

### **Abstracts**

- Krisher, R.L. (2005) Inhibition of the pentose phosphate pathway results in meiotic arrest in porcine oocytes that can be overcome by the addition of pathway cofactors and end products. *Reprod. Fertil. Dev.* 17:293 (abstr. 287).
- Tubman, L., Peter, A., Krisher, R.L. (2005) Effect of energy substrates on metabolism and meiosis of porcine oocytes during in vitro maturation. *Reprod. Fertil. Dev.* 17:301 (abstr. 301).
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- Krisher, R., Wulster-Radcliffe, M., Spurlock, M., Sturek, M. (2006) The Ossabaw Pig as a Model of Polycystic Ovary Syndrome. *Experimental Biology*; abst. 490.3.

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*Illinois:*

**Journal Articles**

Larson, J.E., G.C. Lamb, J.S. Stevenson, S.K. Johnson, M.L. Day, T.W. Geary, D.J. Kesler, J.M. DeJarnette, F.N. Schrick, A. DiCostanzo, and J.D. Arseneau. 2006. Synchronization of estrus in suckled beef cows for detected estrus and artificial insemination and time artificial insemination using gonadotropin-releasing hormone, prostaglandin F<sub>2</sub>, and progesterone. *J. Anim. Sci.* 84:332-342.

Stevenson, J.S., J.R. Pursley, H.A. Garverick, P.M. Fricke, D.J. Kesler, J.S. Ottobre, and M.C. Wiltbank. 2006. Treatment of cycling and noncycling lactating dairy cows with progesterone during ovsynch. *J. Dairy Sci.* 89:2567-2578.

*Wisconsin:*

**Journal Articles**

Silva del Río, N., S. Stewart, P. Rapnicki, Y. M. Chang, and P. M. Fricke. An observational analysis of twin births, calf sex ratio, and calf mortality in Holstein dairy cattle. *J. Dairy Sci.* (In press).

Caraviello, D. Z., K. A. Weigel, P. M. Fricke, M. C. Wiltbank, M. J. Florent, N. B. Cook, K. V. Nordlund, N. R. Zwald, and C. M. Rawson. Survey of management practices related to the reproductive performance of dairy cattle on large commercial farms in the United States. *J. Dairy Sci.* (In press).

Chang, Y. M., O. González-Recio, K. A. Weigel, and P. M. Fricke. Genetic analysis of pregnancy rate with unknown voluntary waiting period in U.S. Holsteins using a threshold model. *J. Dairy Sci.* (In press).

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Caraviello, D. Z., K. A. Weigel, M. Craven, D. Gianola, N. Cook, K. Nordlund, P.M. Fricke, and M.C. Wiltbank. Predicting reproductive performance in Holstein cows using machine learning methods. *J. Dairy Sci.* (In press).

Sterry, R. A., M. L. Welle, and P. M. Fricke. 2006. Treatment with GnRH after first timed AI improves fertility in noncycling lactating dairy cows. *J. Dairy Sci.* (In press).

Roberts, A. J., M. J. Al-Hassan, P. M. Fricke, and S. E. Echterkamp. 2006. Large variation in steroid concentrations and insulin-like growth factor binding proteins exists among individual small antral follicles collected from within cows at various stages of estrous cycle. *J. Anim. Sci.* 84:2714-2724.

Rivera, H., R. A. Sterry, and P. M. Fricke. 2006. Presynchronization with GnRH does not improve fertility to synchronization of ovulation and timed artificial insemination in Holstein heifers. *J. Dairy Sci.* 89:3810-3816.

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- Sterry, R. A., M. L. Welle, and P. M. Fricke. 2006. Effect of interval from timed AI to initiation of resynchronization of ovulation on fertility of lactating dairy cows. *J. Dairy Sci.* 89:2099-2109.
- Wilson, R. D., P. M. Fricke, M. L. Leibfried-Rutledge, J.J. Rutledge, C. M. Syverson Penfield, and K. A. Weigel. 2006. In vitro production of bovine embryos using sex-sorted sperm. *Theriogenology* 65:1007-1015.

### **Refereed Abstracts**

- Sterry, R. A., P. Jardon, B. Ryzebol, and P. M. Fricke. 2006. Effect of timing of the second GnRH injection of a timed AI protocol on fertility of lactating Holstein cows after first postpartum and Resynch AI services. *J. Dairy Sci.* 89(Suppl. 1):49.
- Silva, E. B. P., R. A. Sterry, D. Kolb, N. Mathialagan, M. F. McGrath, J. M. Ballam, and P. M. Fricke. 2006. Accuracy of pregnancy diagnosis in Holstein cows using transrectal ultrasonography based on a serum pregnancy associated glycoprotein (PAG) ELISA. *J. Dairy Sci.* 89(Suppl. 1):204.
- Silva, E. P. B., R. A. Sterry, and P. M. Fricke. 2006. Assessment of a practical method for identifying anovular dairy cows synchronized for first postpartum timed artificial insemination. *J. Dairy Sci.* 89(Suppl. 1):205.
- Fricke, P. M. 2006. Invited: ADSA Southern Section Symposium - Practical and Applied Approaches to Managing Dairy Business in the Future: Future Challenges for Reproductive Management of Dairy Cattle. *J. Dairy Sci.* 89(Suppl. 1):271.
- Watters, R. D., M. C. Wiltbank, P. M. Fricke, J. N. Guenther, A. E. Kulick, and R. R. Grummer. 2006. Effect of dry period duration on reproductive measures during the subsequent lactation in Holstein cows. *J. Dairy Sci.* 89(Suppl. 1):271.
- Jordan, E. R., J. S. Stevenson, P. M. Fricke, and M. Overton. 2006. Invited: ADSA Production Division, Dairy Reproduction Terminology Workshop. *J. Dairy Sci.* 89(Suppl. 1):382.
- Fricke, P. M., R. A. Sterry, and E. P. B. Silva. 2006. Invited: Systematic Synchronization and Resynchronization Systems for Managing Reproduction in Lactating Dairy Cows. Midwest Branch ADSA Meetings, Des Moines, IA, Abstract 279, p. 88-89.
- Sterry, R. A., E. P. B. Silva, D. Kolb, M. L. Welle, and P. M. Fricke. 2006. Administration of GnRH 5 days after first timed artificial insemination improves fertility in anovular lactating dairy cows. Midwest Branch ADSA Meetings, Des Moines, IA, Abstract 209, p. 66.
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- Fricke, P. M. 2006. Synch Programs and Ultrasound – Are We Getting in There Too Early? Proc. Am. Assoc. Bovine Pract., September 23, St. Paul, MN.
- Sterry, R. A., M. L. Welle, and P. M. Fricke. 2006. Post-Breeding Treatments to Improve Fertility in Lactating Dairy Cows, Proc. Am. Assoc. Bovine Pract., September 23, St. Paul, MN.

- Watters, R., P. M. Fricke, M. C. Wiltbank, P. Clark, and R. R. Grummer. 2006. Impact of dry period length. Proc. Four-State Nutrition and Management Conference, June 14, Dubuque, IA. MWPS-4SD19, pp. 49-54.
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*Iowa:*

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- Stevenson JS, Pursley JR, Garverick HA, Fricke PM, Kesler DJ, Ottobre JS, Wiltbank MC. Treatment of cycling and noncycling lactating dairy cows with progesterone during Ovsynch. 2006. *J Dairy Sci*. 897:2567-78.

*South Dakota:*

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- Perry, G. A., and B. L. Perry. 2006. Influence of preovulatory concentrations of estradiol on interval to ovulation and uterine pH. *J. Anim. Sci.* 84(Suppl. 1):149 Abstr. 201
- Nelson, J. R., B. L. Perry, and G. A. Perry. 2006. Effects of standing estrus and concentrations of estradiol on uterine pH. *J. Anim. Sci.* 84(Suppl. 1):451 Abstr. 637
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