

## CONTROL OF ESTRUS AND OVULATION IN BEEF COWS<sup>1</sup>

D.J. Patterson, J.M. Thomas, J.W.C. Locke, E.R. Knickmeyer, R.C. Bonacker, and M.F. Smith

Division of Animal Sciences  
University of Missouri, Columbia, MO

### INTRODUCTION

Estrus synchronization and artificial insemination (AI) remain the most important and widely applicable reproductive biotechnologies available for cattle (Seidel, 1995). Although hormonal treatment of heifers and cows to group estrous cycles has been a commercial reality now for over 30 years, beef producers until now have been slow to adopt this management practice. Perhaps this is because of past failures, which resulted when females that were placed on estrus synchronization treatments failed to reach puberty or to resume normal estrous cycles following calving. In addition, early estrus synchronization programs failed to manage follicular waves, resulting in more days in the synchronized period, which ultimately precluded fixed-time artificial insemination (FTAI) with acceptable pregnancy rates. The development of convenient and economical protocols to synchronize estrus and ovulation to facilitate use of FTAI with resulting high fertility has resulted in increased adoption of these important management practices (Patterson et al., 2003). Research conducted over the past 10 to 15 years has focused on the development of methods that effectively synchronize estrus in postpartum beef cows and replacement beef heifers by decreasing the period of time over which estrus detection is required, thus facilitating the use of FTAI.

Improving traits of major economic importance in beef cattle can be accomplished most rapidly through selection of genetically superior sires and widespread use of artificial insemination. Procedures that facilitate synchronization of estrus in estrous cycling females and induction of an ovulatory estrus in peripubertal heifers and anestrous postpartum cows will increase reproductive rates and expedite genetic progress. Estrus synchronization can be an effective means of increasing the proportion of females that become pregnant early in the breeding period resulting in shorter calving seasons and more uniform calf crops (Dziuk and Bellows, 1983). Females that conceived to a synchronized estrus calved earlier in the calving season and weaned calves that were on average 13 days older and 21 pounds heavier than calves from non-synchronized females (Schafer et al., 1990).

Effective estrus synchronization programs offer the following advantages: 1) cows or heifers are in estrus at a predicted time which facilitates AI, embryo transfer, or other assisted reproductive techniques; 2) the time required for detection of estrus is reduced thus decreasing labor expense

---

<sup>1</sup>**Acknowledgements.** Research summarized in this manuscript was supported by National Research Initiative Competitive Grant no. 00-35203-9175 and 2005-55203-15750 from the USDA National Institute of Food and Agriculture. The authors gratefully acknowledge Zoetis (Florham Park, NJ) for providing Lutalyse and EAZI BREED CIDR Cattle inserts; Merial (Athens, GA) for providing Cystorelin; Bayer Animal Health (Monheim, Germany) for providing OvaCyst; ABS Global, Accelerated Genetics, Genex Cooperative, Inc., and Select Sires, Inc., for providing semen. We express appreciation as well to SL Lock at Genex Cooperative, Inc.; Mason-Knox Ranch, Frankfort, SD; Ogren Angus, Langford, SD; and DS McAtee and JJD Schreffler at the University of Missouri Thompson Research Center for their dedicated support of this research.

associated with estrus detection; 3) AI becomes more practical, enabling producers to utilize genetically superior high accuracy sires; 4) cattle will conceive earlier during the breeding period; and 5) calves will be older and heavier at weaning.

This review focuses on estrus synchronization protocols for beef cows that may be used to facilitate artificial insemination. These procedures include synchronization of estrus in estrous cycling females, and induction of estrus accompanied by ovulation among cows that have not returned to estrus after calving. The following protocols and terms will be referred to throughout this manuscript.

Protocols for AI performed on the basis of detected estrus:

*PG*: Prostaglandin F<sub>2α</sub> (PG; Lutalyse<sup>®</sup>, Estrumate<sup>®</sup>, ProstaMate<sup>®</sup>, InSynch<sup>®</sup>, estroPLAN<sup>®</sup>).

*GnRH-PG (Select Synch)*: Gonadotropin-releasing hormone injection (GnRH; Cystorelin<sup>®</sup>, Factrel<sup>®</sup>, Fertagyl<sup>®</sup>, OvaCyst<sup>®</sup>) followed in 7 days with an injection of PG.

*PG 6-day CIDR*: PG followed in 3 days with an injection of GnRH at CIDR insertion; 6 days later CIDRs are removed coincident with the administration of PG.

Protocols for fixed-time AI:

*7-day CO-Synch + CIDR*: GnRH is administered at CIDR insertion on day 0, followed 7 days later with CIDR removal, and PG. Insemination is performed 66 hours after CIDR removal and PG, with GnRH administered at AI.

*5-day CO-Synch + CIDR*: GnRH is administered at CIDR insertion on day 0, followed 5 days later with CIDR removal, and PG. A second injection of PG is administered 8 ± 2 hours after CIDR removal and the first PG injection. Insemination is performed 72 hours after CIDR removal and the first injection of PG, with GnRH administered at AI.

Terms:

*Estrous response*: The number of females that exhibit estrus during a synchronized period.

*Synchronized period*: The period of time during which estrus is expressed after treatment.

*Synchronized conception rate*: The proportion of females that become pregnant of those that exhibit estrus and are inseminated during the synchronized period.

*Synchronized pregnancy rate*: Proportion of females that become pregnant of the total number treated.

To avoid problems when using estrus synchronization, cows should be selected for a program when the following conditions are met: 1) Adequate time has elapsed from calving to the time synchronization treatments are implemented [in most cases a minimum of 40 days postpartum at the beginning of treatment is suggested]; 2) Cows are in average or above-average body condition [scores of at least 5 on a scale of 1 to 9]; and 3) Cows experience minimal calving problems.

## DEVELOPMENT OF METHODS TO SYNCHRONIZE ESTRUS

The development of methods to control the estrous cycle of the cow has occurred in six distinct phases. The physiological basis for estrus synchronization followed the discovery that progesterone inhibited ovulation (Ulberg et al., 1951) and preovulatory follicular maturation (Nellor and Cole, 1956; Hansel et al., 1961; Lamond, 1964). Regulation of estrous cycles was

believed to be associated with control of the corpus luteum, whose life span and secretory activity are regulated by trophic and lytic mechanisms (Thimonier et al., 1975; Patterson et al., 2003). The Progesterone Phase included efforts to prolong the luteal phase of the estrous cycle or to establish an artificial luteal phase by administering exogenous progesterone. Later, progestational agents were combined with estrogens or gonadotropins in the Progesterone–Estrogen Phase. Prostaglandin F<sub>2α</sub> and its analogs were reported in 1972 to be luteolytic in the bovine (Lauderdale, 1972; Rowson et al., 1972; Liehr et al., 1972; Lauderdale et al., 1974) and ushered in the PG Phase. Treatments that combined progestational agents with PG characterized the Progestogen-PG Phase. All of these protocols addressed control of the luteal phase of the estrous cycle since follicular waves were not recognized at the time.

Precise monitoring of ovarian follicles and corpora lutea over time by transrectal ultrasonography expanded our understanding of the bovine estrous cycle and particularly the change that occurs during a follicular wave (Fortune et al., 1988). Growth of follicles in cattle occurs in distinct wave-like patterns, with new follicular waves occurring approximately every 10 days (6-15 day range). We now know that precise control of estrous cycles requires the manipulation of both follicular waves and luteal lifespan (GnRH-PG Phase).

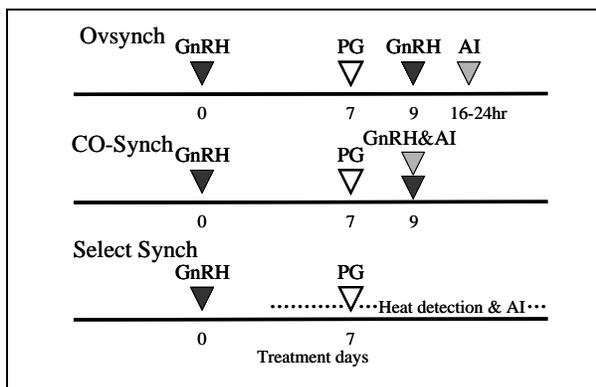
A single injection of gonadotropin-releasing hormone (GnRH) to cows at random stages of their estrous cycles causes release of luteinizing hormone leading to synchronized ovulation or luteinization of most large dominant follicles ( $\geq 10$  mm; Garverick et al., 1980; Bao and Garverick, 1998; Sartori et al., 2001). Consequently, a new follicular wave is initiated in all cows within 2 to 3 days after GnRH administration. Luteal tissue that forms after GnRH administration is capable of undergoing PG-induced luteolysis 6 or 7 days later (Twagiramungu et al., 1995). The GnRH-PG protocol increased estrus synchronization rate in beef (Twagiramungu et al., 1992a,b) and dairy (Thatcher et al., 1993) cattle. A drawback of this method, however, is that approximately 5 to 20% of the cows are detected in estrus on or before the day of PG injection, thus reducing the proportion of females that are detected in estrus and inseminated during the synchronized period (Kojima et al., 2000; Stevenson et al., 2000; DeJarnette et al., 2001a, b). This information stimulated research in the Progestogen-GnRH-PG Phase.

**Synchronizing estrus and ovulation with the GnRH-PG-GnRH protocol.** Administration of PG alone is commonly utilized to synchronize an ovulatory estrus in estrous cycling cows. However, this method is ineffective in anestrus females and variation among animals in the stage of the follicular wave at the time of PG injection directly contributes to the variation in onset of estrus during the synchronized period (Macmillan and Henderson, 1984; Sirois and Fortune, 1988). Consequently, the GnRH-PG-GnRH protocol was developed to synchronize follicular waves and timing of ovulation. The GnRH-PG-GnRH protocol (Figure 1) for fixed-time AI results in development of a preovulatory follicle that ovulates in response to a second GnRH-induced LH surge 48 hours after PG injection (Ovsynch; Pursley et al., 1995). Ovsynch was validated as a reliable means of synchronizing ovulation for fixed-time AI in lactating dairy cows (Pursley et al., 1995; Burke et al., 1996; Pursley et al., 1997a, b; Schmitt et al., 1996). Time of ovulation with Ovsynch occurs between 24 to 32 hours after the second GnRH injection and is synchronized in 87 to 100% of lactating dairy cows (Pursley et al., 1997a). Pregnancy rates among cows that were inseminated at a fixed time following Ovsynch ranged from 32 to 45% (Pursley et al., 1997b;

1998). The Ovsynch protocol, however, did not effectively synchronize estrus and ovulation in dairy heifers (35% pregnancy rate compared with 74% in PG controls; Pursley et al., 1997b).

Protocols for FTAI were then tested in postpartum beef cows. Pregnancy rates for Ovsynch treated beef cows were compared with those of cows synchronized and inseminated at a fixed time following treatment with Syncro-Mate-B (Geary et al., 1998a). Calves in both treatment groups were removed from their dams for a period of 48 hr beginning either at the time of implant removal (Syncro-Mate-B) or at the time PG was administered (Ovsynch). Pregnancy rates following FTAI after Ovsynch (54%) were higher than for Syncro-Mate-B (42%) treated cows. One should note that on the day following FTAI, cows were exposed to fertile bulls of the same breed; no attempt was made to determine progeny paternity. Additionally, we do not know the incidence of short cycles among cows that were anestrus prior to treatment and that perhaps returned to estrus prematurely and became pregnant to natural service.

Variations of the Ovsynch protocol (CO-Synch and Select Synch) were tested in postpartum beef cows (Figure 1). It is important to understand that treatment variations of Ovsynch used in postpartum beef cows did not undergo the same validation process that Ovsynch underwent in lactating dairy cows. At this point we do not know whether response in postpartum beef cows to the protocols outlined in Figure 1 is the same or different from lactating dairy cows due to potential differences in follicular wave patterns. Differences in specific response variables may include: a) the relative length of time to ovulation from the second GnRH injection; b) the anticipated range in timing of ovulation; and c) the degree of ovulation synchrony that occurs. Two variations from Ovsynch used most extensively in postpartum beef cows are referred to as CO-Synch and Select Synch (Figure 1). CO-Synch (Geary et al., 1998b) is similar to Ovsynch in that timing and sequence of injections are the same and all cows are inseminated at a fixed time. CO-Synch differs from Ovsynch, however, in that cows are inseminated when the second GnRH injection is administered, compared to the recommended 16 hours after GnRH for Ovsynch treated cows. Select Synch (Geary et al., 2000) differs too, in that cows do not receive the second injection of GnRH and are not inseminated at a fixed time. Cows synchronized with this protocol are inseminated 12 hours after detected estrus. It is currently recommended for Select Synch treated cows that detection of estrus begin as early as 4 days after GnRH injection and continue through 6 days after PG (Kojima et al., 2000). Select Synch, similar to Ovsynch, was less effective than the melengestrol acetate (MGA)-PG protocol in synchronizing estrus in beef heifers (Stevenson et al., 1999).



**Figure 1.** Methods currently being used to synchronize estrus and ovulation in postpartum beef cows using the GnRH-PG protocol: Ovsynch, CO-Synch and Select Synch.

**IMPORTANT CONSIDERATIONS RELATED TO CHOOSING A PROGESTIN-BASED PROTOCOL  
FOR BEEF HEIFERS OR COWS**

Use of MGA as part of any estrus synchronization protocol in beef cows constitutes an extra-label use of medicated feed that is prohibited by the Animal Medicinal Drug Use and Clarification Act and regulation 21 CFR 530.11(b). The feeding of MGA is specifically approved for estrus suppression in heifers only (Federal Register, 1997). Removal of MGA from the ration allows heifers to return to estrus and be inseminated or bred in a synchronized time. Although 35 years of feeding MGA to beef cows and beef heifers has demonstrated MGA is safe, effective and economical (Zimbelman, 1963; Zimbelman and Smith, 1966; Zimbelman et al., 1970; Brown et al., 1988; Patterson et al., 1989; Patterson et al., 1995; Imwalle et al., 1998; Deutscher, 2000; Lamb et al., 2000; Patterson et al., 2000; Kojima et al., 2000; Patterson et al., 2002; Wood et al., 2001; Perry et al., 2002; Stegner et al., 2004a, b, c; Wood-Follis et al., 2004; Perry et al., 2004; Bader et al., 2005; Schafer et al., 2007; Sá Filho et al., 2009), the feeding of MGA to adult cows is not an FDA approved label claim and therefore is strictly prohibited by the FDA. It is unfortunate that the MGA label does not include all reproductively mature beef cattle, but it does not. The results reported in the proceedings from this conference, regarding use of the CIDR device in beef cows demonstrates that a viable alternative to MGA is available and approved for use by FDA/CVM. Producers that have used MGA to synchronize cows in the past should transition to CIDR to comply with FDA regulations concerning extra-label use of medicated feeds.

**DEVELOPMENT OF THE 7-DAY CIDR-PG PROTOCOL FOR POSTPARTUM BEEF COWS**

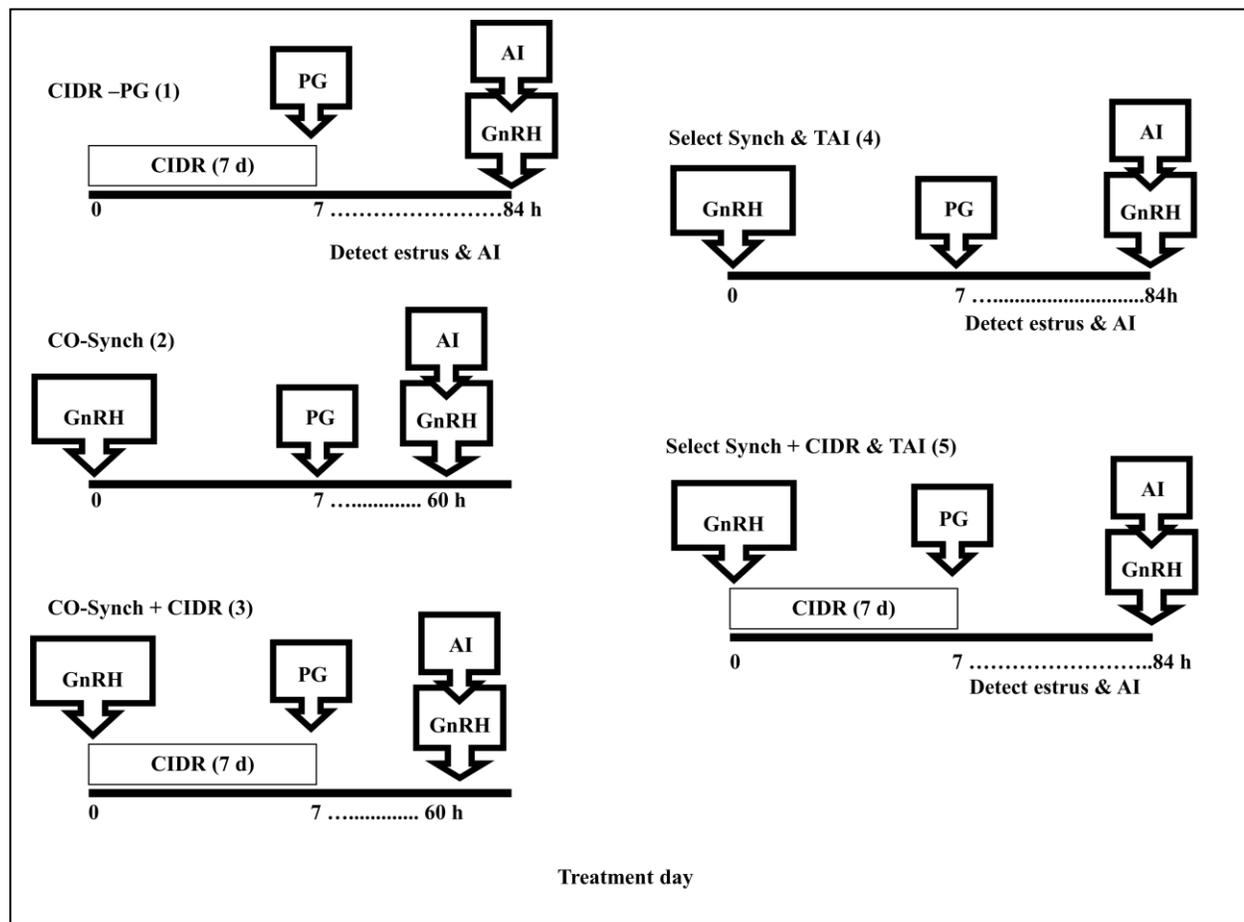
Lucy et al., (2001; Table 1) summarized results from initial studies conducted in the U.S. involving controlled internal drug release (CIDR)-based protocols for use in synchronizing estrus in postpartum beef cows. These data were submitted to FDA in support of the original approval for the CIDR in beef heifers and cows. Three treatments were involved in the study and included: 1) untreated control; 2) PG only; and 3) 7-day CIDR-PG. The 7-day CIDR-PG treated cows had CIDRs inserted for 7 days with PG administered on day 6 of CIDR treatment. The 7-day CIDR-PG protocol yielded greater pregnancy rates compared with control or PG treated cows. Treatment with CIDR increased synchronization rates within the first 3 days following PG, resulting in enhanced pregnancy rates. The improved pregnancy rate in anestrous cows treated with the CIDR was noteworthy because anestrous cows in the control or PG treatments never attained pregnancy rates that were similar to those of the 7-day CIDR-PG treated group. The drawback of the protocol was that PG was administered on day 6 after CIDR insertion, which required an additional day of handling the cows.

**Table 1.** Synchronization, conception, and pregnancy rate for postpartum beef cows (modified from Lucy et al, 2001).

<u>Item</u>	<u>Synchronization rate</u>		<u>Conception rate</u>		<u>Pregnancy rate</u>	
	No.	%	No.	%	No.	%
Anestrous						
Control	16/151	11	6/16	38	6/151	4
PG	30/154	19	17/30	57	17/154	11
CIDR-PG	64/142	45	36/63	57	36/141	26
Cyclic						
Control	26/134	19	15/26	58	15/134	11
PG	63/129	49	44/63	70	44/129	34
CIDR-PG	102/141	72	64/101	63	64/140	46
Total						
Control	42/285	15	21/42	50	21/285	7
PG	93/283	33	61/93	66	61/283	22
CIDR-PG	166/283	59	100/164	61	100/283	35

#### DEVELOPMENT OF THE 7-DAY CO-SYNCH + CIDR PROTOCOL

**The multi-state CIDR trial.** Lamb et al. (2001) and Stevenson et al. (2003) reported that pregnancy rates resulting from AI increased from 48 to 59% with the addition of a CIDR device to the CO-Synch protocol; indicating that supplemental progesterone offered the potential to improve pregnancy rates that resulted after fixed-time AI. Larson et al. (2006; Figure 2) designed a study to determine whether a fixed-time AI protocol could yield pregnancy rates similar to a protocol that required detection of estrus, and whether inclusion of a CIDR to a GnRH-PG based protocol would increase pregnancy rates resulting from AI. The study was conducted with 14 herds in 7 states, and included five experimental treatments shown in Figure 2. Cows assigned to the CIDR-PG, Select Synch & TAI, and Select Synch + CIDR and TAI treatments were observed for signs of behavioral estrus for 3 days up to 72 hours after PG. Cows in each treatment that failed to exhibit estrus by this time were inseminated at 84 hours after PG. Pregnancy rates resulting from AI during the synchronized period are listed in Table 2.



**Figure 2.** Treatment schedules for cows in the multi-state CIDR trial (Larson et al., 2006).

**Table 2.** Pregnancy rates following AI among beef cows in the multi-state CIDR trial.<sup>1</sup>

	Treatments									
	<u>1</u>		<u>2</u>		<u>3</u>		<u>4</u>		<u>5</u>	
	No.	%								
Total	266/506 <sup>x</sup>	53	238/548 <sup>y</sup>	43	290/539 <sup>x</sup>	54	269/507 <sup>x</sup>	53	289/498 <sup>x</sup>	58

<sup>1</sup>Refer to Figure 2 for a description of the 5 treatment protocols.

<sup>x</sup>Percentages without a common superscript letter differ,  $P < 0.05$ .

Larson et al, 2006.

Results listed in Table 2 support previous studies that reported an improvement in fixed-time AI pregnancy rates in postpartum beef cows with the addition of a CIDR to the CO-Synch protocol. These differences may result from the potential added benefit of induced cyclicity among anestrous cows that occurred following CIDR removal. Addition of the CIDR to the CO-Synch protocol prevents premature expression of estrus that occurs between GnRH and PG among cows that do not receive a CIDR. In the absence of a CIDR, it is estimated that 5 to 20% of the total number of cows treated will exhibit estrus prior to and immediately after PG injection (Kojima et al., 2000;

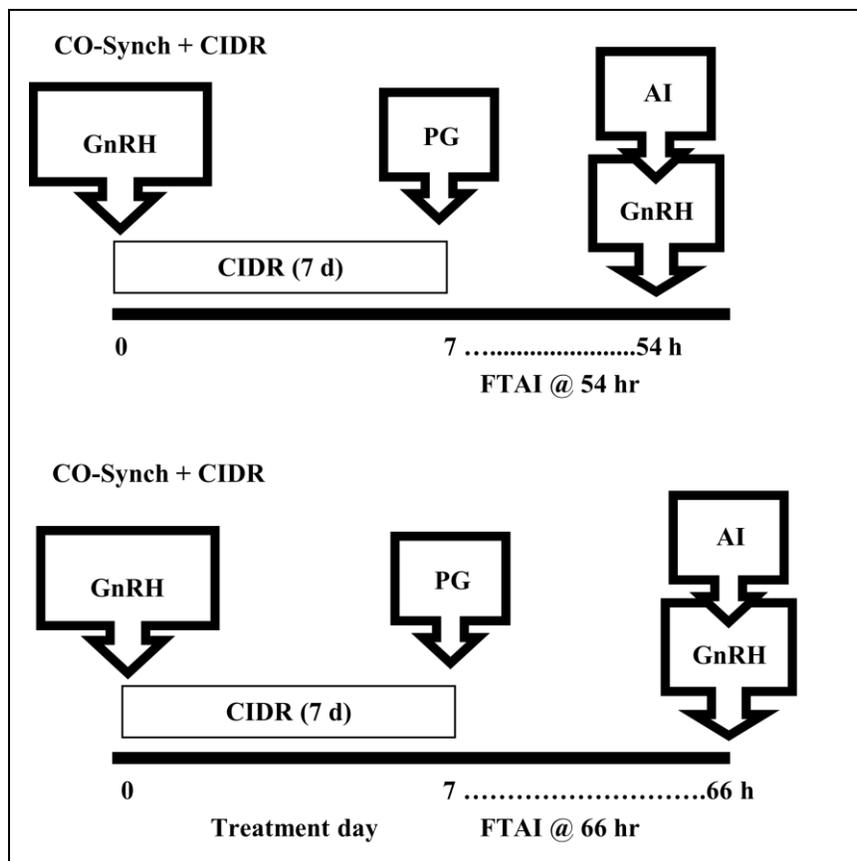
Stevenson et al., 2000; DeJarnette et al., 2001a, b). Therefore, addition of the CIDR to the CO-Synch protocol prevents the premature expression of estrus prior to or following PG.

Larson et al. (2006) reported that pregnancy rates resulting from clean-up AI in the 3 treatments that involved estrous detection ranged from 26 to 39%, which resulted in a 9 to 11% increase in pregnancy rates. These results indicate that when using these protocols, a clean-up time AI is required to achieve pregnancy rates that are similar to those resulting from the CO-Synch + CIDR fixed-time AI protocol that requires no estrous detection. The authors concluded that the Select Synch + CIDR and TAI protocol consistently produced the highest pregnancy rates across the various locations; however the CO-Synch + CIDR protocol was effective in facilitating fixed-time AI without detecting estrus. The studies reported by Lamb et al. (2001) and Larson et al. (2006) indicate that pregnancy rates resulting from fixed-time AI may be expected to differ on the basis of parity, days postpartum, body condition and geographic location.

#### **DOES TIMING OF INSEMINATION AFFECT PREGNANCY RATES RESULTING FROM FIXED-TIME AI FOLLOWING ADMINISTRATION OF THE CO-SYNCH + CIDR PROTOCOL?**

Several studies involving the CO-Synch + CIDR protocol evaluated the effect of timing of AI on subsequent pregnancy rates resulting from FTAI with insemination times varying from 48 to 72 h after PG (Bremer et al., 2004; Dobbins et al., 2006). Timing of insemination following the CO-Synch + CIDR protocol was based on recommendations from the pharmaceutical and AI industries (54 to 66 h) and other reports where the timing of AI included 48, 54, 56, 60, 64, 66, and 72 h post PG (Lamb et al., 2001; Bremer et al., 2004; Dobbins et al., 2006; Larson et al., 2006; Schafer et al., 2007). For this reason, Busch et al. (2008) compared FTAI pregnancy rates among lactating beef cows synchronized with the CO-Synch + CIDR protocol that were inseminated at 54 or 66 h after PG, and characterized the estrous response of cows in each treatment prior to FTAI (Figure 3). In this study, the interval from PG to FTAI (mean  $\pm$  SD) was  $54.2 \pm 0.5$ h and  $66.2 \pm 0.4$ h for cows in the 54 and 66 h treatments, respectively. There was no effect of year, location, technician, sire, or pre-treatment cyclicity status (Table 3) before initiation of treatment on pregnancy rates resulting from FTAI. However, there was a significant effect of treatment (timing of insemination) on pregnancy rates resulting from FTAI among all cows (Table 4). Based on the odds ratio, cows inseminated at 66 h following PG administration were 1.32 times more likely to conceive to the FTAI than cows inseminated at 54 h following PG administration. Final pregnancy rate at the end of the 60 d breeding season did not differ between treatments (Table 4).

Busch et al. (2008) reported that estrous response following PG and prior to FTAI was greater among cows inseminated at 66 h (50%) than cows inseminated at 54 h (26%). However, there was no difference in estrous response within treatment between cyclic and anestrus cows and no overall difference between cyclic and anestrus cows. Mean interval from PG to estrus was shorter for FTAI at 54 h (mean  $\pm$  SE,  $46.7 \pm 1.1$  h) compared to FTAI at 66 h ( $53.8 \pm 1.0$  h); however, these differences occurred as a result of HeatWatch transmitters being removed at AI. Cows that exhibited estrus prior to FTAI had significantly higher pregnancy rates (76%) than cows that did not exhibit estrus before AI (56%; Table 5). Also, cows that exhibited estrus before FTAI at 66 h had a higher pregnancy rate (81%) than cows that exhibited estrus before FTAI at 54 h (65%).



**Figure 3.** Treatment schedule for cows assigned to the CO-Synch + CIDR protocol with FTAI at 54 or 66 h. From Busch et al. (2008).

**Table 3.** Pregnancy rates after fixed-time artificial insemination based on estrous cyclicity before initiation of treatments.<sup>1</sup>

Year	Location	FTAI at 54 h				FTAI at 66 h			
		Cyclic		Anestrus		Cyclic		Anestrus	
		Proportion	%	Proportion	%	Proportion	%	Proportion	%
Year 1	1	50/79	63	16/29	55	49/79	62	17/31	55
	2	52/88	59	7/15	47	67/93	72	5/10	50
	Combined	102/167	61	23/44	52	116/172	67	22/41	54
Year 2	1	28/42	67	39/56	70	35/51	69	36/50	72
	2	48/82	58	17/33	52	64/90	71	13/22	59
	Combined	76/124	61	56/89	63	99/141	70	49/72	68
TOTAL		178/291	61	79/133	59	215/313	69	71/113	63

<sup>1</sup>See Figure 3 for a description of the protocols.  
Busch et al. (2008)

The onset of estrus prior to FTAI in beef cows was shown to improve pregnancy rates when compared to cows that did not exhibit estrus (Perry et al., 2005). Busch et al. (2008) reported that cows that exhibited estrus following administration of the CO-Synch protocol had greater serum estradiol concentrations during the 2 days before insemination compared to cows that were induced to ovulate. Busch et al. (2008) concluded that cows that exhibited estrus may have attained concentrations of estradiol necessary to effectively prepare follicular cells for luteinization, and (or) induced an adequate number of uterine progesterone receptors (Zelinski et al., 1980); thus providing an adequate uterine environment for pregnancy establishment and maintenance. These data indicate that higher estrous response rates prior to FTAI in beef cows should result in greater pregnancy rates resulting from FTAI, provided that AI is performed within an acceptable time period following the peak estrous period. It is important to note that there was no difference within treatment in pregnancy rates resulting from FTAI between cows that were classified as cyclic or anestrus before treatment initiation. These results indicate that the CO-Synch + CIDR protocol effectively induces cyclicity in anestrus cows as measured by estrus, ovulation, and pregnancy outcome.

A key observation from the study by Busch et al. (2008) lies in the fact that pregnancy rates resulting from fixed-time AI at 66 h following administration of the CO-Synch + CIDR protocol were consistent across locations and years. These results too, as they relate to pregnancy rates following fixed-time AI, compare favorably with the study by Schafer et al. (2007) and from field trials in Missouri reported by Patterson et al. (2007). Interestingly, too, these results are consistently higher than those reported in the literature (Lamb et al., 2001; Bremer et al., 2004; Dobbins et al., 2006; Larson et al., 2006) when cows were inseminated at 48, 56, 60, 64 or 72 h following treatment administration, all of which point to the importance of timing of insemination and the associated impact on pregnancy outcome.

**Table 4.** Pregnancy rates of cows in response to fixed-time AI and at the end of the breeding season.<sup>1</sup> From Busch et al. (2008).

Year	Location	Pregnancy rate to fixed-time AI <sup>1</sup>		Pregnancy rate at end of breeding season <sup>2</sup>	
		Proportion	%	Proportion	%
Year 1	Combined				
	FTAI at 54 h	125/211	59	183/209	88
	FTAI at 66 h	138/213	65	199/212	94
Year 2	Combined				
	FTAI at 54 h	132/213	62	202/213	95
	FTAI at 66 h	148/213	69	190/212	90
TOTAL	FTAI at 54 h	257/424	61 <sup>x</sup>	385/422	91
TOTAL	FTAI at 66 h	286/426	67 <sup>y</sup>	389/424	92

<sup>1</sup>See Figure 3 for a description of the protocols.

<sup>2</sup>Pregnancy rate to fixed-time AI determined by ultrasound 56 to 78 d after AI.

<sup>3</sup>Pregnancy rate at the end of the breeding season determined 60 to 110 d after the end of a 60 d breeding season.

<sup>x,y</sup>Means within a column with different superscripts are different,  $P = 0.05$ .

**Table 5.** Pregnancy rate based on estrous response (Location 2; Busch et al., 2008).

Estrous response	<u>54 h</u>		<u>66 h</u>		<u>Combined</u>	
	<u>Proportion</u>	<u>%</u>	<u>Proportion</u>	<u>%</u>	Proportion	%
Exhibited estrus	37/57	65% <sup>x</sup>	86/106	81% <sup>a,y</sup>	123/163	76% <sup>a</sup>
DID NOT exhibit estrus	87/161	54%	63/109	58% <sup>b</sup>	150/270	56% <sup>b</sup>

<sup>1</sup>See Figure 3 for a description of the protocols.

<sup>a,b</sup> Percentage within a column with different superscripts are different,  $P < 0.01$ .

<sup>x,y</sup> Percentage within a row with different superscripts are different,  $P = 0.03$ .

#### DEVELOPMENT OF THE 5-DAY CO-SYNCH + CIDR PROTOCOL

Labor required to detect estrus has limited use of AI in U.S. beef herds (NAHMS, 1998). Consequently, estrus synchronization protocols must not only be effective, but practical and economical to implement. Recently, estrus synchronization protocols were developed that eliminate the need to detect estrus, allowing AI to be performed at predetermined fixed times without reducing AI pregnancy rates (Geary et al., 1998a; Perry et al., 2002; Stegner et al., 2004b; Bader et al., 2005; Larson et al., 2006; Schafer et al., 2007; Busch et al., 2008; Bridges et al., 2008).

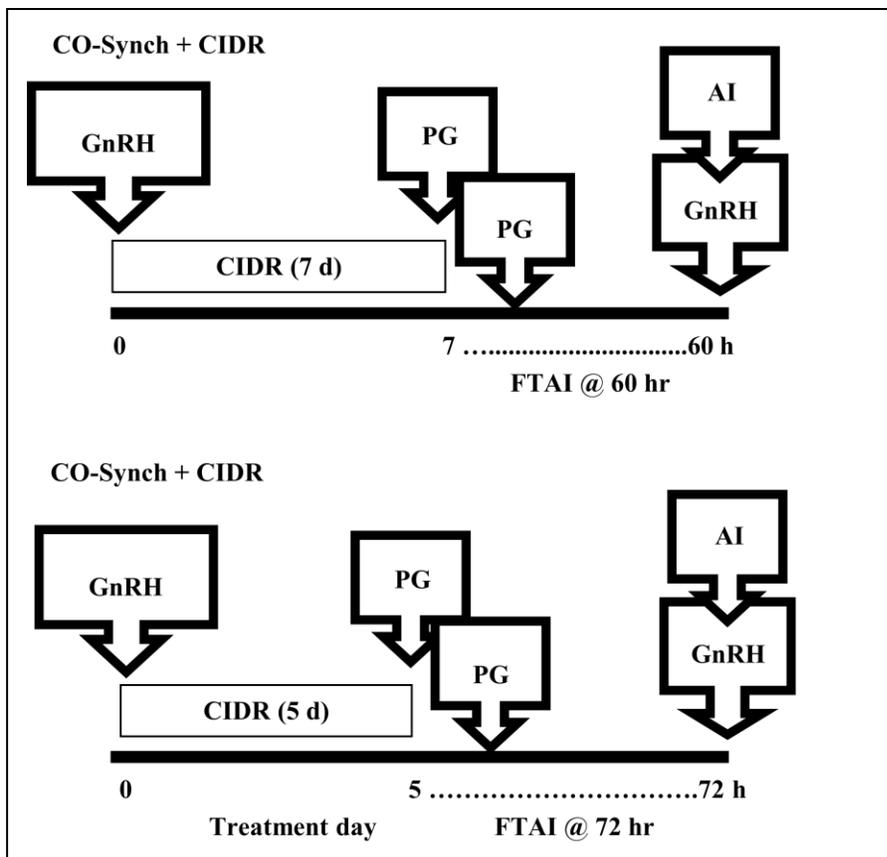
The CO-Synch protocol uses GnRH and PG to synchronize estrus and ovulation prior to FTAI (Geary et al., 1998a; Perry et al., 2002; Lamb et al., 2001; Larson et al., 2006). The 7-d interval between GnRH and PG administration provides the necessary time for a new follicular wave to be recruited, along with selection and maturation of the dominant follicle to a point where successful ovulation of a healthy oocyte may be achieved (Thatcher et al., 1989; Pursley et al., 1995). The 7-day interval also allows time for accessory CL to become responsive to the luteolytic action of a single PG injection.

The CO-Synch protocol was shown to have marginal success in synchronizing estrus before FTAI due to 5 to 15% of estrous cycling cows expressing estrus prior to the time PG was administered (Twagiramungu et al., 1995; Kojima et al., 2000). Addition of the CIDR to the CO-Synch protocol prevents cows from exhibiting estrus prior to the time PG is administered and resulted in enhanced fertility among anestrus cows (Lamb et al., 2001; Larson et al., 2006). Pregnancy rates resulting from FTAI were improved when progestins were used to presynchronize estrus (Perry et al., 2002; Stegner et al., 2004b; Bader et al., 2005; Schafer et al., 2007) prior to the administration of GnRH and PG or were included as part of the CO-Synch protocol (Lamb et al., 2001; Larson et al., 2006; Schafer et al., 2007; Busch et al., 2008).

Bridges et al. (2008) hypothesized that a modification to the 7-day CO-Synch + CIDR protocol would conceptually increase secretion of estradiol by the ovulatory follicle and decrease the incidence of induced ovulation of follicles with reduced estrogenic activity. Bridges et al. (2008) proposed that shortening the duration of CIDR treatment with a 5-day protocol would result in

improvements in FTAI pregnancy rates. This hypothesis was based on the premise that day 4 dominant follicles have higher intrafollicular concentrations of estradiol-17 $\beta$  (E<sub>2</sub>) and a greater ability to produce E<sub>2</sub> compared to older age follicles (Valdez et al., 2005). Bridges et al. (2009) reported that maximum preovulatory concentrations of estradiol tended to be greater in 5-day compared to 7-day CIDR-treated cows that failed to respond to GnRH at CIDR insertion, and that postovulatory circulating concentrations of progesterone were greater among 5-day compared to the 7-day treated cows. Increased follicular concentrations of E<sub>2</sub> and elevated postovulatory concentrations of progesterone are believed to reflect greater physiological maturity of the dominant follicle and to result in higher AI pregnancy rates (Lopez et al., 2005; Perry et al., 2005). Therefore, Bridges et al. (2008) proposed that if CIDR removal and AI are more accurately timed with the 5-day protocol to coincide with follicular development, higher AI pregnancy rates may be achieved.

Bridges et al. (2008) compared the 5-day CO-Synch + CIDR protocol with fixed-time AI 72 hours after CIDR removal and PG with a 7-day CO-Synch + CIDR protocol with fixed-time AI performed 60 hours after CIDR removal and PG (Figure 4). Cows in both treatment groups were administered a second injection of PG 12 hours after CIDR removal and the first PG injection.



**Figure 4.** Cows in the 7-day CO-Synch + CIDR group received GnRH and CIDR inserts on day 0, PG at CIDR removal and again 12 hr later. FTAI was performed at 60 hr after CIDR removal for cows assigned to the 7-day treatment. Cows in the 5-day CO-Synch + CIDR group received GnRH and a CIDR insert on day 0, PG at CIDR removal and again 12 hr later. FTAI was performed at 60 hr after CIDR removal for cows assigned to the 7-day treatment. From Bridges et al. (2008).

**Table 6.** Pregnancy rates of cows after fixed-time AI in the study by Bridges et al. (2008)<sup>1</sup>.

	Experiment 1		Experiment 2		Combined	
	5-day	7-day	5-day	7-day	5-day	7-day
No. cows	105	111	199	201	304	312
FTAI pregnancy rate	80% <sup>x</sup>	67% <sup>y</sup>	65% <sup>x</sup>	56% <sup>y</sup>	70% <sup>x</sup>	60% <sup>y</sup>

<sup>1</sup>See Figure 4 for a description of the protocols.

<sup>x,y</sup> Percentages within a row with different superscripts are different,  $P < 0.05$ .

Pregnancy rates resulting from FTAI for cows assigned to each of the two treatments (Figure 4) are listed in Table 6, and were higher among cows assigned to the 5-day protocol. It is important to point out that cows in both treatments were administered two injections of PG, one at CIDR removal and again 12 hours later. Two injections of PG are required with the 5-day protocol to effectively regress accessory corpora lutea that form as a result of GnRH-induced ovulations at the initiation of treatment (Bridges et al., 2008; Kasimanickam et al., 2008). Kasimanickam et al. (2008) reported that the increase in pregnancy rate associated with a second PG treatment was approximately 15% higher compared with pregnancy rates of cows that received only a single PG injection following administration of the 5-day CO-Synch + CIDR protocol. It is important to point out however, that two injections of PG following CIDR removal are not required for cows assigned to a 7-day protocol. Additionally, cows assigned to the 5-day protocol were inseminated 72 hours after the first injection of PG, whereas cows assigned to the 7-day protocol were inseminated 60 hours after the first PG injection. Bridges et al. (2008) concluded that reducing the interval from GnRH and CIDR insertion from 7 to 5 days, administering two injections of PG at CIDR removal and again 12 hours later, and extending the interval from CIDR removal to FTAI to 72 hours was an effective estrus synchronization protocol for use in facilitating FTAI in postpartum beef cows.

Bridges et al. (2012) reported results from an experiment to determine whether two injections of PG administered at CIDR removal would be an effective alternative to administering two injections at separate time intervals (8 hours apart) for cows assigned to a 5-day CO-Synch + CIDR protocol. Cows in 13 herds and 8 states were involved in the study which compared pregnancy rates among cows assigned to one of three treatment groups: one 25 mg injection of PG administered at CIDR removal and a second 25 mg injection of PG administered 8 hours later; two 25 mg injections of PG administered into two injection sites coincident with CIDR removal; and a single 25 mg injection of PG administered at CIDR removal. Pregnancy rates resulting from FTAI were highest (55%) for cows administered one 25 mg injection of PG at CIDR removal and a second 25 mg injection of PG administered 8 hours later. Pregnancy rates for cows that received two 25 mg injections of PG administered into two injection sites coincident with CIDR removal were intermediate (51%) and not different from the two other treatment groups (single injection, 48%). Based on these data the recommendation for use of this protocol to facilitate fixed-time AI in beef cows is to administer two doses of PG 8 h apart, the first coincident with the time of CIDR removal and the second 8 h later.

**COMPARISON OF THE 7-DAY AND 5-DAY CO-SYNCH + CIDR PROTOCOLS IN FACILITATING FIXED-TIME ARTIFICIAL INSEMINATION IN POSTPARTUM BEEF COWS**

Wilson et al. (2010) conducted two experiments to: 1) Characterize and compare the 7-day and 5-day Select Synch + CIDR protocols on the basis of follicular dynamics and timing and synchrony of estrus following treatment administration (Figure 5); and 2) Compare pregnancy rates resulting from FTAI following administration of the 7-day and 5-day CO-Synch + CIDR protocols with insemination performed 66 and 72 h following treatment, respectively (Figure 7).

In the first experiment, Wilson et al. (2010) reported that response to GnRH, dominant follicle size at GnRH, interval to estrus, variance for interval to estrus, or estrous response did not differ between the 5-day and 7-day Select Synch + CIDR treatments (Table 7). Figure 6 illustrates the distribution of estrus for the two treatments. There was, however, an effect of pre-treatment estrous cyclicity status on estrous response, with 75/82 (92%) of the estrous cycling cows exhibiting estrus and 21/35 (60%) of the anestrus cows exhibiting estrus during the synchronized period. There was no effect of technician or treatment on synchronized conception or pregnancy rates resulting from AI, respectively (Table 8). Pregnancy rates resulting from AI were affected by estrous cyclicity status of cows prior to treatment initiation, with 57/82 (69.5%) of the estrous cycling cows and 12/35 (34.2%) of the anestrus cows conceiving to AI.

**Table 7.** Estrous response, interval to estrus, response to GnRH, and mean follicle size at GnRH.

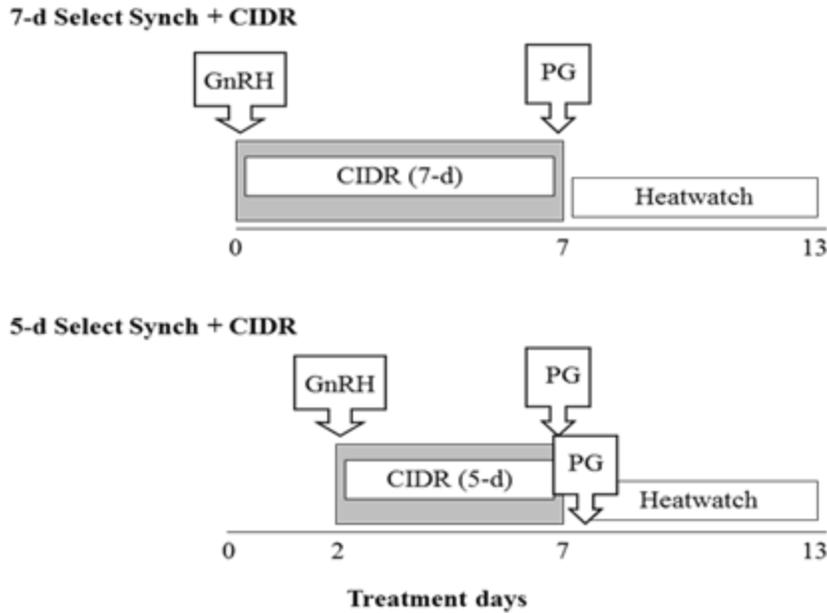
Treatment <sup>1</sup>	Estrous <sup>2</sup> response Proportion (%)		Interval <sup>3</sup> to estrus (h)	Variance for interval to estrus	Response <sup>4</sup> to GnRH Proportion (%)	Dominant follicle size at GnRH (mm)
5-day Select Synch + CIDR	46/58	79	71.2 ± 2.6	316	41/58 71	11.9 ± 0.4
7-day Select Synch + CIDR	50/59	85	64.8 ± 2.6	348	42/59 71	11.9 ± 0.4

<sup>1</sup> See Figure 5 for a description of the treatment protocols. From Wilson et al. (2010).

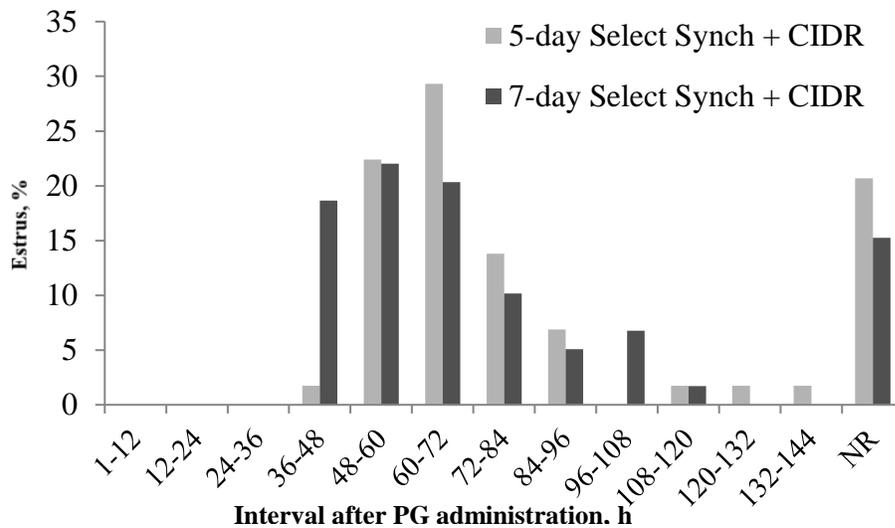
<sup>2</sup> Estrous response = number of cows that exhibited estrus during the synchronized period (0 to 144 h after PG administration).

<sup>3</sup> Interval to estrus = number of hours from administration of PG to onset of estrus (mean ± SE).

<sup>4</sup> Cows were considered to have responded to GnRH if the dominant follicle observed by ultrasonography on day 0 or 1 was no longer visible on day 2 or 3 for the respective treatments (mean ± SE).



**Figure 5.** Cows in the 7-day Select-Synch + CIDR treatment received GnRH and CIDR inserts on day 0. Prostaglandin  $F_{2\alpha}$  was administered and CIDR inserts were removed on day 7. Estrus detection and AI were performed within a 144 h synchronized period after PG was administered. Cows in the 5-day Select-Synch + CIDR treatment received GnRH and a CIDR insert on day 0. Prostaglandin  $F_{2\alpha}$  was administered and CIDR inserts were removed on day 5. A second injection of PG was administered 12 h after the first PG injection. Estrus detection and AI were performed within a 144 h synchronized period after PG. From Wilson et al. (2010).



**Figure 6.** Percentage of cows in the 5-day and 7-day Select Synchrony + CIDR treatments that exhibited estrus after PG: 5-day Select Synchrony + CIDR (gray bar) and 7-day Select Synchrony + CIDR (black bar). NR = no estrous response. See Figure 5 for a description of the treatment protocols. From Wilson et al. (2010).

**Table 8.** Synchronized AI conception rates, synchronized AI pregnancy rates, and final pregnancy rates.

Treatment <sup>1</sup>	Synchronized AI conception rate <sup>2</sup>		Synchronized AI pregnancy rate <sup>3</sup>		Final pregnancy rate <sup>4</sup>	
	Proportion	(%)	Proportion	(%)	Proportion	(%)
5-day Select Synch + CIDR	33/46	72	33/58	57	52/58	90
7-day Select Synch + CIDR	36/50	72	36/59	61	51/59	86

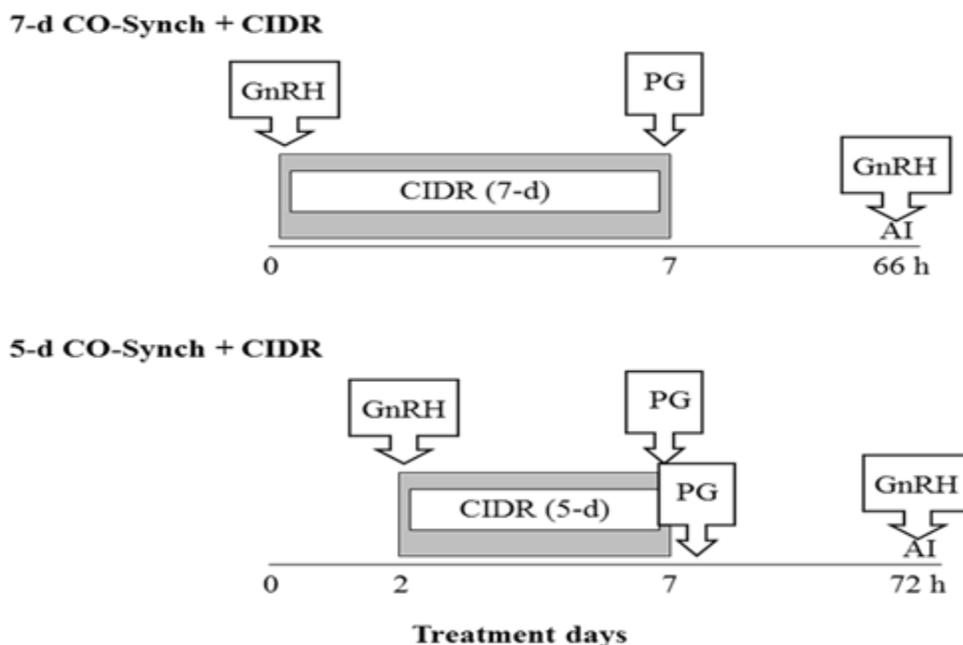
<sup>1</sup> See Figure 5 for a description of the treatment protocols. From Wilson et al. (2010).

<sup>2</sup> Synchronized AI conception rate = number of cows that conceived of those exhibiting estrus and inseminated during the synchronized period (0 to 144 h after PG administration).

<sup>3</sup> Synchronized pregnancy rate = number of cows that conceived of the total number treated during the synchronized period (0 to 144 h after PG administration).

<sup>4</sup> Final pregnancy rate = number of cows that conceived by the end of the 60 day breeding season.

Wilson et al. (2010) designed a second experiment (Figure 7) to compare pregnancy rates resulting from FTAI following administration of the 7-d and 5-d CO-Synch + CIDR protocols with insemination performed 66 and 72 h following treatment, respectively. There were no differences in pregnancy rates resulting from fixed-time AI between the 7-d and 5-d treatment groups, with 67% of the cows in each treatment conceiving to FTAI (Table 9). The interval from PG to FTAI was  $66.4 \pm 1.2$  h and  $72.6 \pm 0.6$ h for the 7-day and 5-day treatment groups, respectively. Estrous cyclicity status prior to treatment initiation had no effect on pregnancy rates resulting from FTAI for cows assigned to the two treatments (Table 10).



**Figure 7.** Cows in the 7-day CO-Synch + CIDR treatment received GnRH and CIDR inserts on day 0. Prostaglandin F<sub>2α</sub> was administered and CIDR inserts were removed on day 7. All 7-day treated cows were fixed-time inseminated 66 h following treatment with GnRH administered at AI. Cows in the 5-day CO-Synch + CIDR treatment received GnRH and a CIDR insert on day 0. Prostaglandin F<sub>2α</sub> was administered and CIDR inserts were removed on day 5. A second injection of PG was administered 12 h after the first PG injection. All 5-day treated cows were fixed-time inseminated 72 h following treatment with GnRH administered at AI. From Wilson et al. (2010).

**Table 9.** Pregnancy rates after fixed-time AI and at the end of the breeding season.

Location and treatment <sup>1</sup>	Pregnancy rate to fixed-time AI		Pregnancy rate at the end of the breeding season	
	Proportion	%	Proportion	%
Location 1				
5d	36/60	60	57/60	95
7d	38/58	66	57/58	98
Location 2				
5d	72/100	72	99/100	99
7d	73/102	72	97/102	95
Location 3				
5d	32/50	64	45/50	90
7d	29/49	59	46/49	94
Overall				
5d	140/210	67	201/210	96
7d	140/209	67	200/209	96

<sup>1</sup> See Figure 7 for a description of the treatment protocols. From Wilson et al. (2010).

**Table 10.** Pregnancy rates after fixed-time AI based on estrous cyclicity before initiation of treatments.

Location	5-d CO-Synch + CIDR <sup>1</sup>				7-d CO-Synch + CIDR <sup>1</sup>			
	Estrous cycling		Anestrus		Estrous cycling		Anestrus	
	Proportion	%	Proportion	%	Proportion	%	Proportion	%
1	34/55	62	2/5	40	35/53	66	3/5	60
2	31/38	82	41/62	66	27/41	66	46/61	75
3	20/29	69	12/21	57	20/34	59	9/15	60
Combined	85/122	70	55/88	63	82/128	64	58/81	72

<sup>1</sup> See Figure 7 for a description of the treatment protocols. From Wilson et al. (2010).

The 5-day and 7-day CO-Synch + CIDR protocols are similar except for differences in the interval from GnRH to PG administration and the length of CIDR treatment (5 versus 7 days, respectively), and that two injections of PG are required with the 5-day protocol. Additionally, FTAI in Wilson's study was performed at 72 h from the first injection of PG for the 5-day treated cows versus 66 h for the 7-day treated cows. Two injections of PG are required with the 5-day protocol to effectively regress accessory corpora lutea that form as a result of GnRH-induced ovulations at the initiation of treatment (Bridges et al., 2008; Kasimanickam et al., 2008). Kasimanickam et al. (2008) demonstrated that cows assigned to the 5-day protocol that received only one injection of PG had significantly lower pregnancy rates resulting from FTAI compared to cows assigned to the 5-day protocol that received two injections of PG 12 h apart. As previously discussed, Bridges et al. (2008) hypothesized that shortening the duration of CIDR treatment from 7- to 5-day would better time CIDR removal coincident with optimal follicular development, and that lengthening the period of proestrus (the time between PG and AI) would result in higher pregnancy rates following FTAI.

Bridges et al. (2008) reported that pregnancy rates following FTAI were higher for cows assigned to the 5-day protocol compared to cows that were assigned to a 7-day protocol. However, the results reported by Wilson et al. (2010) indicate that there were no differences in pregnancy rates resulting from FTAI between 5-day and 7-day treated cows. It is important to contrast these results with those reported by Bridges et al. (2008) for cows assigned to the 7-day protocol. Cows assigned to the 7-day protocol in Wilson's experiment received a single injection of PG and were inseminated 66 h after PG; whereas, 7-day treated cows in Bridges' study received two injections of PG with AI performed 60 h after the first PG injection.

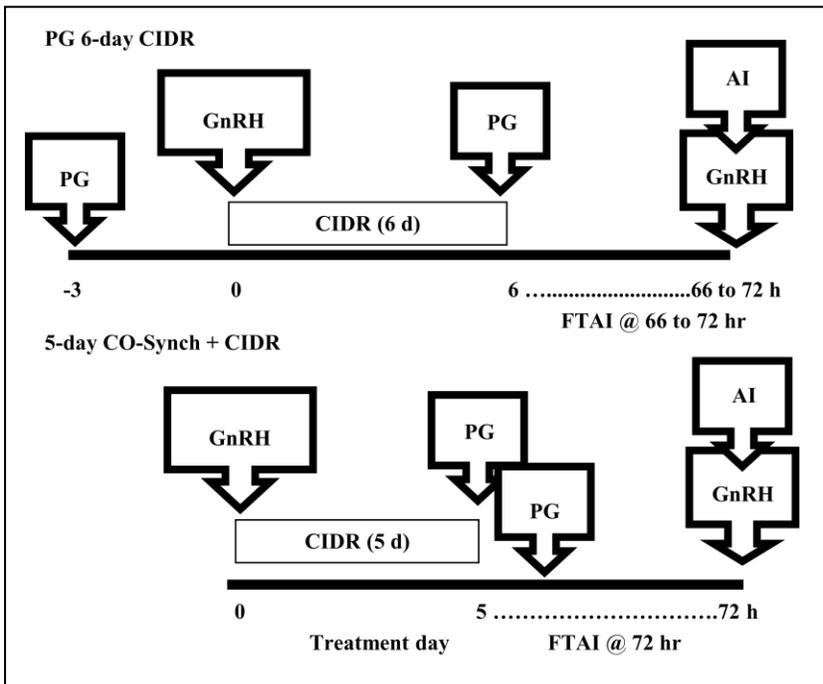
A possible explanation for differences in results between the second experiment reported by Wilson et al. (2010) compared to results reported by Bridges et al. (2008) may be related to the differences in timing of AI for the 7-day treated cows in each study. In comparing results from Experiments 1 and 2 in the studies reported by Wilson et al. (2010) it is important to point out that timing of insemination for the respective protocols in Experiment 2 paralleled the timing of peak estrus from Experiment 1. For cows assigned to the 5-day protocol (Wilson et al., 2010), the mean time of AI (72.6 h) in Experiment 2, paralleled the mean interval to estrus (71.2 h) from Experiment 1. Likewise for the 7-day treated cows (Wilson et al., 2010), the mean time of AI (66.5 h) in Experiment 2 paralleled the mean interval to estrus (64.8 h) reported in Experiment 1. Arguably, lengthening the proestrus period to 66 h for cows assigned to the 7-day protocol may explain the similarity in FTAI pregnancy rates between treatment groups, versus those previously reported by Bridges et al. (2008), in which cows were inseminated at 60 h.

These data (Wilson et al., 2010) suggest that pregnancy rates resulting from FTAI are perhaps more a function of properly timed AI in relation to proestrus, rather than timing CIDR removal and follicular development. Timing of AI is important relative to pregnancy outcome following FTAI. Busch et al. (2008) confirmed that FTAI pregnancy rates were higher when AI was performed at 66 h versus 54 h after administration of the 7-day CO-Synch + CIDR protocol. The 66 h interval would appear to be the near optimal timing of AI based on the results from Wilson et al. (2010); however, no direct comparison of pregnancy rates resulting from AI performed at 60 h versus 66 h has been made. In addition, AI is recommended to be performed 72 h following administration of the 5-day protocol (Bridges et al., 2008), which also coincides with what would be considered to be near the optimal timing of AI from the results reported by Wilson et al. (2010). As previously stated, when pregnancy rates following FTAI for the 5-day and 7-day CO-Synch + CIDR protocols were compared, no differences were found between treatments when cows were inseminated at 72 and 66 h, respectively. These data seem to confirm the premise that length of the proestrus period is an important consideration relative to pregnancy outcome following administration of both the 5-day and 7-day protocols.

Finally, Wilson et al. (2010) and Whittier et al. (2013) compared the 5- and 7-day CO-Synch + CIDR protocols on the basis of pregnancy outcome and practical application in the field. The results from these studies demonstrate that the two treatments performed comparably on the basis of pregnancy rates resulting from FTAI, although the study reported by Whittier et al. (2013) showed a 3% improvement in pregnancy rate resulting from FTAI among cows assigned to the 5-day protocol. Given this observation, the 5-day protocol provides an effective alternative to the 7-day protocol for use in facilitating FTAI, however beef producers must consider the increased labor and treatment costs associated with the 5-day CO-Synch + CIDR protocol.

**DEVELOPMENT OF THE PG 6-DAY CIDR PROTOCOL**

Perry et al. (2012) designed an experiment (Figure 8) to determine whether controlling follicular development to optimize follicle size and estradiol exposure would be effective in maximizing pregnancy success. The objectives of the studies (Perry et al., 2012) were to determine: 1) if inducing luteal regression 3 days prior to an injection of GnRH improved control of follicular turnover, and 2) if inducing luteal regression 3 days before a modified CIDR protocol improved pregnancy success compared to the 5-day CIDR protocol. Results reported from the study (Perry et al., 2012) indicate that induction of luteal regression 3 days before initiation of a modified CIDR protocol increased pregnancy rates compared to the 5-day CIDR protocol. Additionally, induction of luteal regression 3 days prior to the injection of GnRH at the initiation of the PG-CIDR protocol resulted in increased incidence of ovulation following the injection of GnRH. These authors (Perry et al., 2012) suggest that better control of follicular development at the initiation of the CIDR protocol may result in greater pregnancy success. Pregnancy rates resulting from fixed-time AI for the PG 6-day CIDR treated group were higher (160/248, 65%) than the 5-day CO-Synch + CIDR treated group (136/249, 55%). Both protocols require that cows be handled 4 times to effectively administer the complete treatment schedule.



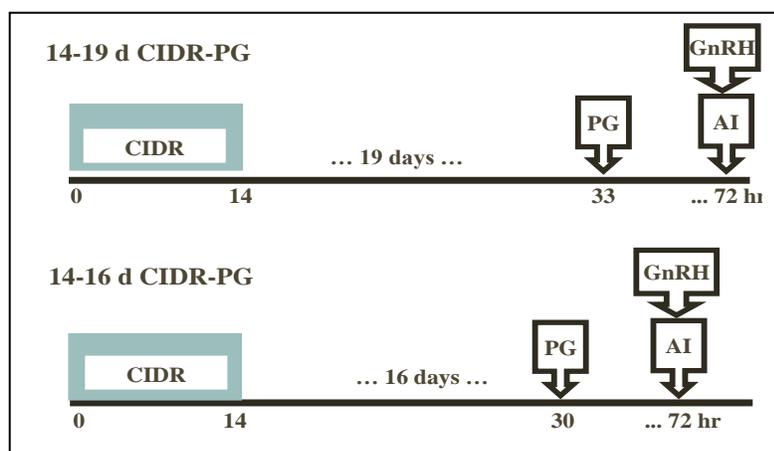
**Figure 8.** Comparison of the PG 6-day CIDR protocol with the 5-day CO-Synch + CIDR protocol (Perry et al., 2012).

**EVALUATING THE 14-DAY CIDR-PG PROTOCOL IN POSTPARTUM BEEF COWS**

Long-term progestin (14-d) administration has been used effectively to synchronize estrus and ovulation in beef heifers and cows (Schafer et al., 2007; Leitman et al., 2009; Mallory et al., 2011; Nash et al., 2012; Nash et al., 2013). Long-term CIDR administration for 14-d suppresses estrus, inhibits ovulation, induces the development of a persistent dominant follicle that can ovulate after CIDR removal, and induces estrous cyclicity in pre- or peripubertal beef heifers and anestrus postpartum beef cows (Ahmad et al., 1995; Revah and Butler, 1996; Roche et al., 1999; Perry et

al., 2004; Leitman et al., 2008). Ovulation of a dominant follicle after CIDR removal creates a synchronized estrous cycle that pre-synchronizes cows prior to FTAI (Nash et al., 2013). In these cases, PG was administered 16 d after CIDR removal with GnRH administered at FTAI. The 14-d CIDR-PG protocol was developed as an alternative to the 14-d melengestrol acetate (MGA)-PG protocol for use in beef heifers (Brown et al., 1988). Deutscher et al. (2000) and Lamb et al. (2000) reported that extending the interval by 2 d from MGA withdrawal to PG improved total estrous response, synchrony of estrus after PG, and resulting pregnancy rates after the synchronized estrous period. Speculation regarding these improvements may pertain to differences in preovulatory follicle size at the time of PG-induced luteolysis, which perhaps stems from differences in follicular dynamics in relation to stage of a follicular wave at the time PG is administered. Nash et al. (2012) reported that pregnancy rates resulting from FTAI were similar for cows assigned to a 14-day CIDR-PG protocol compared to the 7-day CO-Synch + CIDR protocol, however estrous response after PG and prior to FTAI was reduced in cows assigned to the long-term (23%; 14-d CIDR-PG) compared to the short-term protocol (49%; 7-d CO-Synch + CIDR; Nash et al., 2012).

Martin et al. (2014) conducted an experiment (Figure 9) to determine whether extending the interval from CIDR removal to PG among cows assigned to a 14-d CIDR-PG protocol would increase estrous response after PG and potentiate improvements in pregnancy rates after FTAI. The results from this study (Martin et al., 2014) provide evidence that a 14-19 d CIDR-PG schedule may be a more appropriate schedule for mature aged cows  $\geq 4$  yr versus younger aged females (2- and 3-year-olds). Results from this study support the proposed hypothesis that extending the interval from CIDR removal to PG would increase estrous response after PG and potentiate improvements in pregnancy rates after FTAI. Long-term CIDR-based protocols require that cows are handled one extra time compared to short-term schedules (7-d CO-Synch + CIDR). However, this schedule provides the opportunity to combine animal health and reproduction in a single management step, by administering pre-breeding vaccinations at the time of CIDR insertion. The results reported by Martin et al. (2014) agree with previous studies (Nash et al., 2012; Nash et al., 2013) indicating that long-term CIDR-based protocols provide an alternative method of synchronizing estrus prior to FTAI in postpartum beef cows, while at the same time provide labor-reducing management options pertaining to animal health-related considerations.



**Figure 9.** Comparison of a 16- versus a 19-day interval between controlled internal drug release removal and prostaglandin F<sub>2α</sub> following a 14-day controlled internal drug release treatment and fixed-time artificial insemination in postpartum beef cows (Martin et al., 2014).

**HOW DO THE 14-DAY CIDR-PG AND 7-DAY CO-SYNCH + CIDR PROTOCOLS COMPARE IN PRIMIPAROUS 2-YEAR-OLD BEEF COWS?**

Abel et al. (2017) designed a study to compare short- and long-term CIDR-based protocols to synchronize estrus and ovulation in primiparous 2-year-old beef cows. The study was designed to coincide with the recent increase in heifer retention and the significant number of younger age females that are now entering the U.S. cow herd. The objective of the study was to evaluate protocols on the basis of pregnancy rates resulting from FTAI and pregnancy status at the end of the breeding season. Abel et al. (2017) proposed that extended progesterone exposure using the 14-d CIDR-PG protocol would improve pregnancy rates to FTAI and final pregnancy rate at the end of the breeding season by increasing the proportion of females that conceive early in the breeding season. The study (Abel et al., 2017) compared the 14-d CIDR-PG (14-d; n = 355) and 7-d CO-Synch + CIDR (7-d; n = 349) protocols on the basis of estrous response following PG up to the point of FTAI, pregnancy rates after fixed-time AI, and final pregnancy rate. Estrous response prior to FTAI was higher for 7-d compared to 14-d treated cows (Table 11; 74% vs 43%, respectively;  $P < 0.0001$ ); however, pregnancy rates resulting from FTAI were similar (Table 11; 14-d 63%; 7-d 64%;  $P = 0.52$ ). Despite the significant difference in estrous response between cows assigned to each of these protocols there was no difference in pregnancy rate between groups after FTAI. Low estrous response rates were observed in previous studies that evaluated long-term CIDR-based protocols in mixed age groups of beef cows; however, despite the low numbers of cows that exhibited estrus, pregnancy rates resulting from FTAI were acceptable (Nash et al., 2013; Martin et al., 2014). Final pregnancy rates at the end of the breeding season were similar between treatments with 87% and 88% of 14-d and 7-d treated cows conceiving within the first 30 days of the breeding season (Abel et al., 2017; Table 11). These data provide further evidence that progestin-based estrus synchronization protocols afford the potential to increase the proportion of females that conceive early in the breeding season and contribute to reduced culling rates due to reproductive failure. It is worth noting that among a subset of cows in this study, circulating concentrations of  $E_2$  at FTAI were higher ( $P < 0.001$ ) among 14-d CIDR-PG treated cows (8.0 pg/ml, 14-d CIDR-PG; 4.2 pg/ml, 7-d CO-Synch + CIDR) and higher too, among estrous ( $P < 0.004$ ) and non-estrous cows ( $P < 0.05$ ) compared to the 7-d treated cows. Higher concentrations of  $E_2$ , despite smaller dominant follicle diameters among 14-d treated cows, suggest that dominant follicles of cows within this treatment group may be in an active growth stage compared to 7-d treated cows in which growth of the dominant follicle has already plateaued (Abel et al., 2017). These data and results from this study support the concept that pre-ovulatory follicles among cows assigned to the 14-d protocol were physiologically mature at the time GnRH was administered and FTAI was performed. Furthermore, these data help to explain the similarity in pregnancy rate in the face of reduced estrous response among cows assigned to the 14-d CIDR-PG protocol compared to those in the 7-d CO-Synch + CIDR treatment.

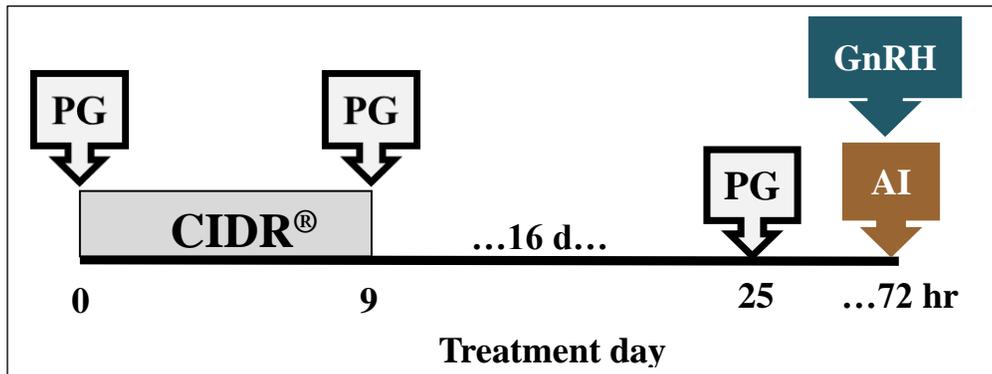
**Table 11.** Estrous response and pregnancy rates of primiparous 2-year-old beef cows.

Response variable	14-day CIDR-PG		7-day CO-Synch + CIDR	
	Proportion	%	Proportion	%
Estrous response	152/355	43 <sup>a</sup>	259/349	74 <sup>b</sup>
FTAI pregnancy rate	223/355	63	222/349	64
Pregnant within 30 d	308/355	87	307/349	88
Pregnant by end of breeding season	339/355	95	334/349	96

<sup>a,b</sup>Estrous response rates within row differ,  $P < 0.0001$ ; From Abel et al., 2017.

### DEVELOPMENT OF THE 9-D CIDR-PG PROTOCOL

Although the 14-d CIDR-PG protocol can be used effectively for mature cows with either a 16 or 19 d interval from CIDR removal to PG, producers may be reluctant to adopt this protocol due to (1) the length of the treatment schedule and (2) low rates of estrous response observed when FTAI is performed at 72 h after PG. To address these considerations, a modified presynchronization approach was developed in which PG is administered at CIDR insertion and removal. Inducing luteolysis among responsive cows in this manner facilitated a shortened duration of progestin treatment from 14 to 9 d while achieving similar size dominant follicles at CIDR removal. Administration of an additional dose of PG at CIDR removal ensures all cows undergo luteolysis, including those cows whose CL may not have responded to the PG administered at CIDR insertion (e.g. cows who had ovulated < 5 d prior). As in the standard 14-d CIDR-PG protocol, PG is administered 16 d after CIDR removal. Preliminary results (Thomas et al., 2016a) indicated a tendency for increased estrous response rate after CIDR removal following the 9-d CIDR-PG treatment schedule compared to the 14-d CIDR-PG. In addition, estrous response after PG was significantly enhanced among 9-d CIDR-PG treated cows. Split-time AI was conducted, with cows having expressed estrus receiving timed AI at 72 h after PG, and cows not expressing estrus by 72 h receiving timed AI 24 h later at 96 h. In this pilot study, a tendency ( $P = 0.09$ ) was observed for higher pregnancy rates among 9-d CIDR-PG treated cows (77%) compared to 14-d CIDR-PG treated cows (60%). A subsequent field trial (Thomas et al., 2017) with larger numbers evaluated the 9-d CIDR-PG versus 14-d CIDR-PG protocols with respect to endocrine parameters, ovarian dynamics, and pregnancy rate to FTAI performed at 72 h following PG administration. Serum estradiol concentrations, follicle size, and estrous response did not differ based on treatment. However, cows assigned to the 9-d CIDR-PG protocol (Figure 10) tended to achieve greater FTAI pregnancy rates than cows assigned to the 14-d CIDR-PG protocol (62% versus 52%;  $P = 0.07$ ).



**Figure 10.** Treatment schedule for the 9-d CIDR-PG protocol. Prostaglandin  $F_{2\alpha}$  (PG) is administered concurrent with CIDR insertion on Day 0 and CIDR removal on Day 9. On Day 25, PG is administered again, and FTAI is performed 72 h after PG administration on Day 28. Gonadotropin-releasing hormone (GnRH; 100  $\mu$ g im) is administered to all cows concurrent with FTAI (Thomas et al., 2016a; Thomas et al., 2017).

### **SPLIT-TIME AI: DELAYED INSEMINATION OF NON-ESTROUS BEEF COWS IN FTAI**

The elimination of estrus detection from FTAI protocols greatly decreases the labor and inconvenience of carrying out an AI program, and as a result, increasing numbers of beef cows are being inseminated by appointment (Thomas et al., 2014a,b). However, previous studies report varying pregnancy rates to FTAI based on estrus expression prior to FTAI, with females that expressed estrus performing significantly better than females that failed to express estrus prior to FTAI (Busch et al., 2007; Busch et al., 2008; Wilson et al., 2010; Mallory et al., 2011; Mallory et al., 2013; Perry and Smith, 2015). This is particularly problematic when using sex-sorted semen, as conception to sex-sorted semen is currently maximized when cows are inseminated following an observed estrus (Seidel, 2007). Appointment breeding without estrus detection is currently not recommended with sex-sorted semen (Seidel, 2011). Sex-sorted semen presents unique challenges for use in FTAI both in terms of sperm cell quantity and quality. Fewer sperm cells are placed in an AI dose of sex-sorted semen (typically  $2 \times 10^6$  cells per dose as compared to  $20 \times 10^6$  cells per dose in conventional semen), and lower sperm cell number per dose has been demonstrated to negatively impact fertility (Den Daas et al., 1998; DeJarnette et al., 2008). Sex-sorted semen is also characterized by decreased sperm quality. Hollinshead et al. (2003) found that the sex-sorting process induces a higher proportion of capacitated sperm cells, and this and other stresses of the cell sorting process may limit the fertile lifespan of sperm cells which have been sex-sorted. Therefore, insemination closer to the time of ovulation is generally advantageous.

Thomas et al. (2014a) found that delaying insemination of non-estrous cows until 20 h after GnRH administration, a strategy termed "split-time AI," improved pregnancy rates when using sex-sorted semen in conjunction with FTAI. Estrus was synchronized for 656 suckled beef cows with the 7-day CO-Synch + CIDR protocol. Estrus detection aids (Estroject) were applied at PG and CIDR removal on d 7, and estrous expression was recorded at GnRH on d 10. Cows were assigned to one of three treatments: (1) FTAI (concurrent with GnRH, 66 h after CIDR removal) with conventional semen regardless of estrous expression; (2) FTAI with sex-sorted semen regardless of estrous expression; or (3) FTAI with sex-sorted semen for cows having expressed estrus, and delayed AI 20 h after final GnRH for cows failing to express estrus. Higher pregnancy rates ( $P < 0.0001$ ) were achieved with conventional semen (Treatment 1, 77%) than with sex-sorted semen (Treatments 2 and 3, 51% and 42%, respectively) among cows that expressed estrus. However, among cows that failed to express estrus, delayed insemination with sex-sorted semen yielded higher ( $P < 0.0001$ ) pregnancy rates than with sex-sorted semen at the standard time (Treatments 2 and 3, 3% versus 36%, respectively). Furthermore, among cows that failed to express estrus, FTAI pregnancy rates when using sex-sorted semen at the delayed time (36%) were comparable ( $P = 0.9$ ) to those achieved using conventional semen at the standard time (Treatment 1; 37%). These results indicate that delaying insemination of non-estrous cows by 20 h from the standard FTAI improved pregnancy rates when sex-sorted semen is used with FTAI.

Thomas et al. (2014b) also evaluated this approach when using conventional, non-sex-sorted semen. Estrus was synchronized for 951 mature, suckled beef cows across 9 locations using the 7-d CO-Synch + CIDR protocol. Cows within each location were assigned to one of two treatments based on age, days postpartum (DPP), and body condition score (BCS): 1) FTAI (concurrent with GnRH, 66 h after PG) regardless of estrous expression or; 2) FTAI for cows expressing estrus, and delayed AI (20 h after GnRH) for cows failing to express estrus. The results from this field trial

showed no significant effect of treatment on AI pregnancy rate ( $P = .76$ ).

Insemination at the optimal time relative to estrus may be more or less critical based on individual bull fertility (Macmillan and Watson, 1975). It was hypothesized that delaying insemination of non-estrous females until 20 h after GnRH administration would better align the lifespan of viable, capacitated sperm with the timing of ovulation, potentially allowing for higher FTAI pregnancy rates. This effect may occur in certain situations with respect to optimizing the timing of sperm fertility, such as when using sex-sorted semen. However, the results from Thomas et al. (2014b) suggest that development of a favorable female environment may be the more critical factor in increasing the pregnancy rates of non-estrous females receiving delayed insemination.

Estrus expression prior to AI is associated with improved fertility for a number of reasons. Initiation of estrus in cattle is preceded by a rise in serum concentrations of estradiol (Allrich, 1994; Perry and Smith, 2015), and preovulatory estradiol coordinates several physiological processes that contribute to the establishment and maintenance of pregnancy, including effects on follicular cells, the oocyte, gamete transport, and preparation of the uterine environment (Pohler et al., 2012). Lower estradiol concentrations among non-estrous females may also affect pregnancy rates by impairing sperm transport in the female reproductive tract. Perry et al. (2005) suggested that preovulatory concentrations of estradiol during standing estrus may modulate uterine pH to affect sperm transport. Delaying insemination of non-estrous cows allowed time for initiation of estrus among a larger percentage of these cows, and the resulting increase in pregnancy rates of the estrous cows may be attributed to a more favorable uterine and oviductal environment. When receiving delayed insemination 20 h after GnRH administration, cows that expressed estrus during the 20 h delay period achieved significantly higher pregnancy rates than those cows that still had not expressed estrus by the time of delayed insemination (67% versus 40%; Thomas et al., 2014b).

Bishop et al. (2016) designed an experiment to evaluate timing of GnRH administration in beef cows based on estrous status with split-time AI. Estrus was synchronized for 622 cows across six locations using the 7-d CO-Synch + CIDR protocol. Estrous detection aids (Estroject) were applied at CIDR removal and PG on d 7, with estrus recorded at 66 and 90 h after PG on d 10 and 11, respectively. Timing of insemination was based on expression of estrus 66 h after PG. Cows in each treatment that exhibited estrus by 66 h were inseminated; whereas AI was delayed 24 h until 90 h after PG for cows failing to exhibit estrus by 66 h. Cows in treatment 1 were administered GnRH 66 h after PG irrespective of estrus expression; whereas in treatment 2, cows were administered GnRH coincident with delayed insemination only if non-estrous at 66 h after PG. Treatment affected overall estrous response (1=85%; 2=90%;  $P=0.04$ ) but did not affect total AI pregnancy rate (1=58%; 2=57%;  $P=0.89$ ). There were no differences between treatments in estrous response at 66 h (1=73%; 2=75%;  $P=0.47$ ); and pregnancy rate resulting from AI for cows inseminated at 66 h was not influenced by GnRH (1=63%; 2=59%;  $P=0.51$ ). Estrous response during the 24 h delay period differed between treatments (1=45%; 2=61%  $P=0.04$ ), although AI pregnancy rate for cows inseminated at 90 h did not differ (1=44%; 2=49%;  $P=0.51$ ). This study indicated that when split-time AI is used in conjunction with the 7 day CO-Synch + CIDR protocol, administration of GnRH at AI to cows that exhibit estrus by 66 h after PG is not warranted.

Bishop et al. (2017) then designed an experiment to compare pregnancy rates in postpartum beef cows following split-time or fixed-time artificial insemination. Estrus was synchronized for 671 cows following administration of the 7-d CO-Synch + CIDR protocol. Cows assigned to the FTAI

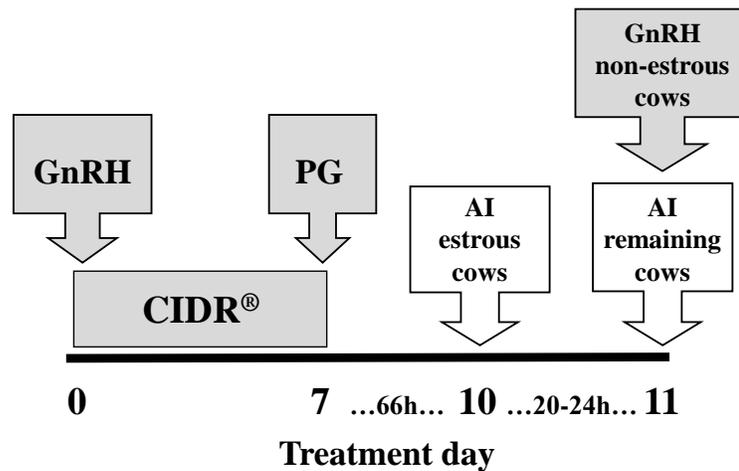
treatment (n = 333; FTAI) were inseminated at 66 h after PG and GnRH was administered concurrent with insemination regardless of estrus expression. For cows in the split-time AI treatment (n = 338; STAI), STAI was performed at 66 and 90 h after PG, and estrus was recorded at these times. Cows in the STAI treatment that exhibited estrus by 66 h were inseminated at that time and did not receive GnRH, whereas AI was delayed 24 h until 90 h after PG for cows that failed to exhibit estrus by 66 h. Gonadotropin-releasing hormone was administered concurrent with AI at 90 h only to cows failing to exhibit estrus. Estrus expression that occurred during the 24 delay period among cows assigned to the STAI treatment increased the total proportion of cows that expressed estrus prior to insemination (FTAI = 60%; STAI = 86%;  $P < 0.001$ ). Pregnancy rates for cows inseminated at 66 h that exhibited estrus did not differ between treatments (FTAI = 58%; STAI = 58%;  $P = 0.93$ ); however, pregnancy rates among non-estrous cows at 66 h was improved (FTAI = 35%; STAI = 51%;  $P = 0.01$ ) among cows assigned to the STAI treatment when insemination was postponed by 24 h. Consequently, total AI pregnancy rate tended to be higher for cows that received STAI (FTAI = 49%; STAI = 56%;  $P = 0.06$ ). In summary, following administration of the 7-d CO-Synch + CIDR protocol, total estrous response increased and pregnancy rates resulting from AI tended to be higher among cows assigned to STAI versus FTAI treatments (Bishop et al., 2017; Figure 11).

Bishop et al. (2017) reviewed other studies reported in the literature involving STAI that generally concurred with results reported above, however inconsistency in the design of STAI experiments makes it difficult to compare the overall outcome and resulting interpretation regarding the utility of STAI in cows. In a series of experiments reported by Hill et al. (2016a,b), all cows that exhibited estrus prior to the initial insemination time were assigned to a control treatment or separate treatments that did not correspond to treatments for cows failing to exhibit estrus prior to that time point. The way in which these experiments were designed precluded any meaningful evaluation of overall pregnancy rates for STAI in either experiment and results were reported separately for estrous and non-estrous cows at the initial insemination. In a subsequent experiment, Markwood et al. (2014) established two insemination times (58 and 76 h) and recorded estrous status of cows at the time AI was performed, however cows were not inseminated at the different time points based on estrus expression. A comparison of pregnancy rate of estrous cows at 58 h to non-estrous cows at 76 h yielded a tendency for overall increased pregnancy rate compared to all cows inseminated in the experiment. However, this was not a preassigned treatment and was not significant when compared to the timed AI pregnancy rate achieved by cows at either 58 or 76 h.

These STAI trials also differ from the study reported by Bishop et al. (2017) in that initial inseminations were performed at 58 or 60 h rather than at 66 h, which raises multiple questions. First, insemination during the peak of estrus onset may result in a variable number of cows being inseminated at the first AI, especially if the number of cows at different locations influences the time interval when estrous status is evaluated. The published estrus onset distributions for cows following estrus synchronization using the 7-d CO-Synch + CIDR protocol indicate peak onset of estrus occurred from 48-60 h following PG (Wilson et al., 2010). In addition, inseminating cows at or near the onset of estrus requires that sperm remain viable in the cow's reproductive tract until the time of ovulation 24-32 hours later (Pursley et al., 1995). Busch et al. (2008) compared pregnancy rates to timed AI at 54 and 66 h after PG following estrus synchronization using the 7-d CO-Synch + CIDR protocol and found that overall AI pregnancy rate was improved by inseminating at 66 h rather than 54 h. More importantly, cows that exhibited estrus prior to

insemination achieved a higher pregnancy rate to AI if inseminated at 66 h (81% at 66 h vs. 65% at 54 h), and the later insemination time allowed more cows to exhibit estrus prior to insemination (50% vs 26%). Overall, an initial insemination time for estrous cows that is earlier rather than later may reduce AI pregnancy rates for this subset of cows. This could artificially inflate the effect of delaying insemination for non-estrous cows, but ultimately decreases overall pregnancy rates when utilizing STAI.

**Using estrus detection aids to facilitate split-time AI.** To identify animals that have not expressed estrus, EstroTECT® estrus detection aids can be applied at the time of the final PG administration. EstroTECT® patches are designed with a scratch-off coating that is removed progressively as an estrous animal stands to be mounted. Because of this design, producers can have a high degree of confidence about the estrus status of females. When using split-time AI, an animal is considered as having expressed estrus when over 50% of the scratch-off coating has been removed from the EstroTECT® patch (Thomas et al., 2016b; Bishop et al., 2017; Figure 11).



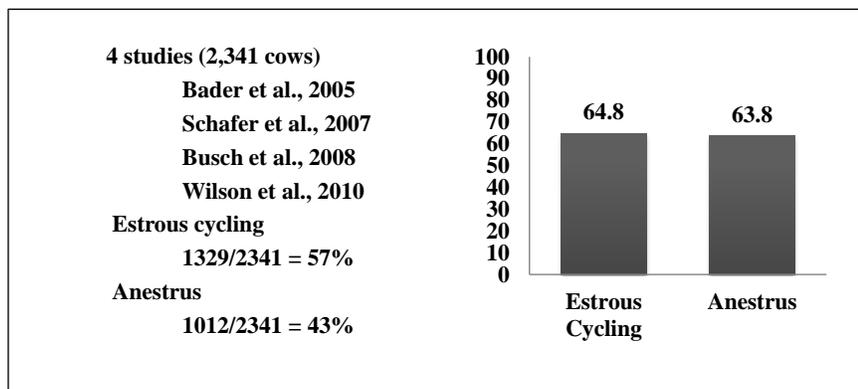
**Figure 11.** The 7-day CO-Synch + CIDR protocol for cows with split-time AI (Thomas et al., 2016b; Bishop et al., 2017).

#### MANAGEMENT CONSIDERATIONS RELATED TO ESTRUS SYNCHRONIZATION AND FTAI

**Managing anestrus.** As previously discussed, one of the main challenges herd owners face at the beginning of any breeding period, is the number of cows that have resumed estrous cyclicity following calving and before the breeding season begins. A primary advantage in administering a CIDR-based protocol in postpartum beef cows lies in the fact that these protocols not only facilitate estrous synchronization and AI in cyclic cows, but offer the added benefit of induced estrous cyclicity in anestrus cows following treatment administration. Figure 12 highlights results from four published studies conducted by our laboratory comparing pregnancy rates after fixed-time AI. In each of these studies, postpartum beef cows were blood sampled twice before placement on an estrus synchronization protocol to determine pretreatment estrous cyclicity status. Results were then compared on the basis of efficacy of the protocols in facilitating FTAI, and evaluating how

cows that were anestrus prior to treatment administration compared to cyclic cows on the basis of pregnancy outcome after FTAI. The combined results from these four studies clearly demonstrate the significant benefit associated with treatment with a progestin prior to FTAI, as there was only a 1 percentage point difference in pregnancy rates among cows that were cyclic or anestrus prior to treatment administration.

**Figure 12.** Pregnancy rates (% pregnant) after fixed-time AI in postpartum beef cows based on pretreatment estrous cyclicity status.



**What to expect at calving.** Numerous studies support the use of estrus synchronization not only as a means of facilitating rapid genetic improvement of beef herds, but perhaps, more importantly, as a powerful reproductive management tool. Profitability may be increased by reducing the extent to which labor is required during the calving period, and increasing the pounds of calf weaned that result from a more concentrated calving distribution and a resulting increase in the age of calves at weaning. Cumulative calving distribution patterns indicate that in many cases over 85% of pregnant cows among synchronized herds will calve within the first 30 days of the calving period (Perry et al., 2002 ; Stegner et al., 2004a,b,c; Bader et al., 2005; Schafer et al., 2007; Busch et al., 2008; Wilson et al., 2010).

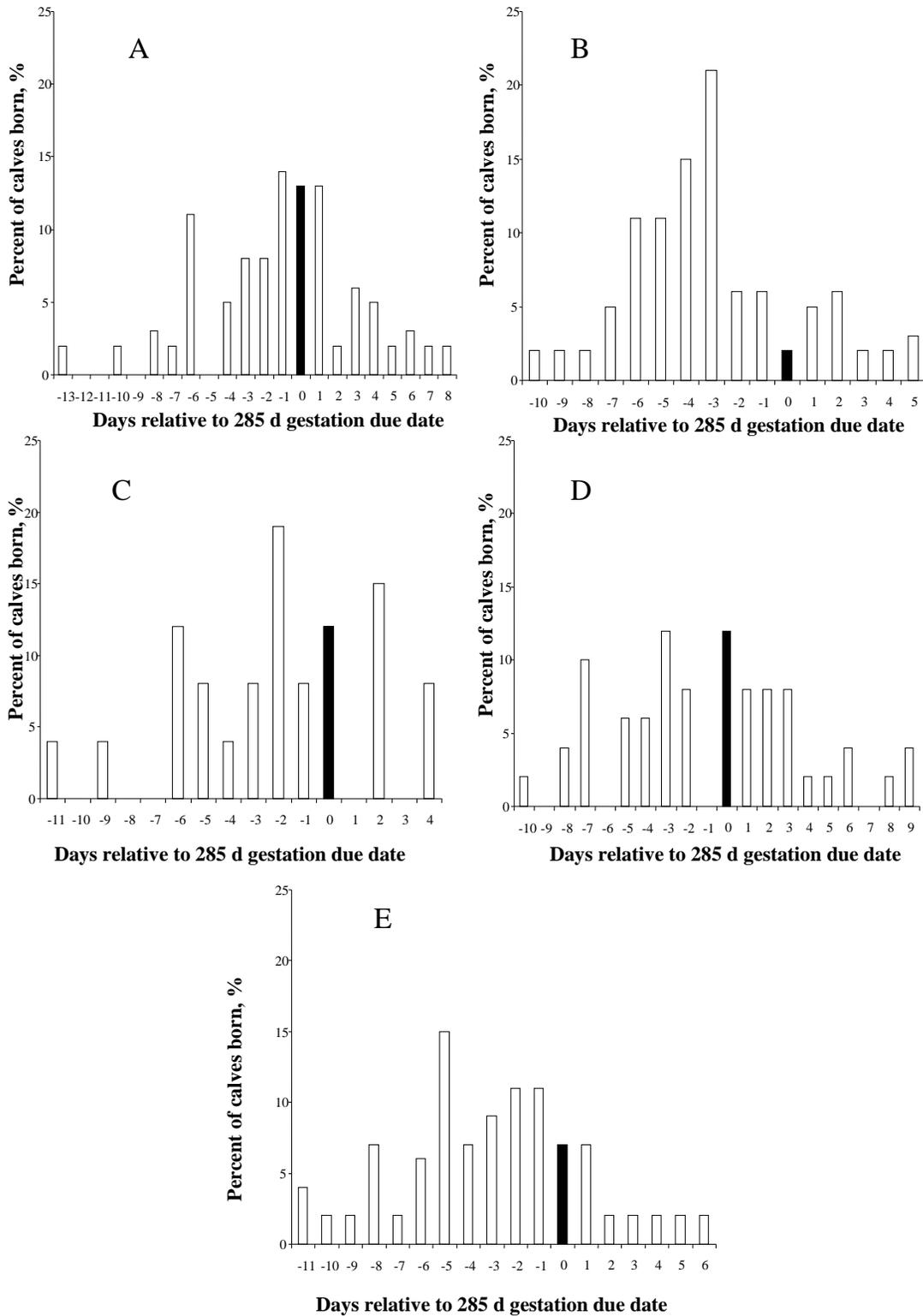
Calving dates for cows that conceived on the same day to fixed-time AI were recorded to address concerns that pertain to the subsequent calving period (Bader et al., 2005). Calf birth dates were recorded for cows that conceived to fixed-time AI at each location involved in the study by Bader et al. (2005). The resulting calving distribution for cows that conceived to the respective sires at each of the locations in the two treatments is illustrated in Figure 13. Calving distribution patterns differed among individual sires (Table 12;  $P < 0.05$ ).

Calving distribution among cows that conceived to fixed-time AI for Location 1 (sires A and B) was 21 and 16 days, respectively. Distributions for Location 2 (sires C and D) were 16 and 20 days, respectively. The calving distribution among cows at location 3 (sire E), was 18 days. Sire B at Location 1 and sire E at Location 3 was the same sire. Cows that conceived on the same day gave birth to calves over a 16 to 21 day period, dependent upon the respective sire. These distributions indicate that successful use of FTAI will not result in an overwhelming number of cows calving on the same day(s). This furthermore suggests that current management practices will not need to be greatly altered to accommodate the early portion of the calving season.

**Table 12.** Comparison of gestation lengths (Mean  $\pm$  SE) among AI sires and locations.

Location	Sire	Gestation length, days	Range, days
1	A	283.5 $\pm$ 0.5	272 - 292
	B <sup>a</sup>	282.1 $\pm$ 0.5	275 - 290
2	C	282.9 $\pm$ 0.8	274 - 289
	D	284.1 $\pm$ 0.6	275 - 294
3	E <sup>a</sup>	282.0 $\pm$ 0.5	274 - 291

<sup>a</sup>Sire B at location 1 and sire E at location 3 are the same sire.  
From Bader et al. (2005).

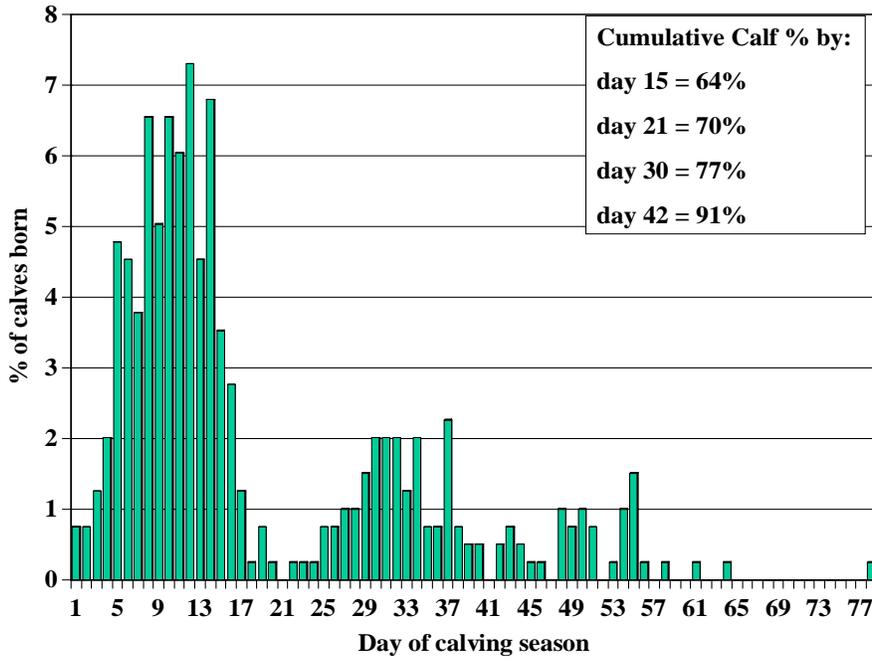


**Figure 13.** Calving distribution patterns at the respective locations for cows that conceived to fixed-time AI. Calving dates among cows that conceived on the same day to the respective sires (A, B, C, D, and E) were 21, 16, 16, 20, and 18 days. Sire B at Location 1 and sire E at Location 3 were the same sire. The shaded bar in each graph represents an anticipated 285 day gestation due date. From Bader et al. (2005).

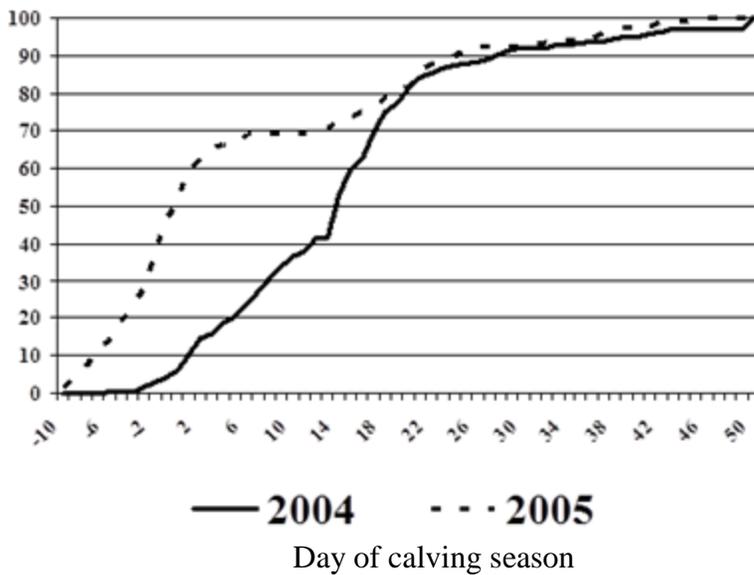
**Consider the impact of estrus synchronization on calving distribution.** Economic considerations related to use of estrus synchronization and choice of the various protocols to use in beef heifers and cows was reviewed by Johnson and Jones (2004). Hughes (2005) reported that opportunities to increase profits for cow-calf operations lie in managing females from the later calving intervals forward toward the first and second 21-day calving intervals. Hughes (2005) reports that added pounds are the economic reward to tightening up the calving interval. The CHAPS benchmark values utilize IRM-SPA guidelines for operating high production herds. These guidelines suggest that 61% of the calves within a herd should be born by day 21 of the calving period, 85% by day 42, and 94% by day 63. Hughes (2005) goes on to say that today's high market prices are generating big economic rewards to intensified management, but more specifically "management as usual" may be what is amiss for many cow calf producers.

Figure 14 illustrates the combined calving data for 3 of 4 locations in the study reported by Schafer (2005). Data from the fourth location was not included in the summary since cows that failed to conceive to AI were sold prior to the calving period. It is interesting to note that in comparison to the recommendation by Hughes (2005), 64% of the cows in this study had calved by day 15, 70% by day 21, 77% by day 30, and 91% by day 42. The economic reward for improvements in calf weaning weight that result from an increase in calf age at weaning, in many cases may offset the cost of implementing estrus synchronization in beef herds.

Finally, Figure 15 illustrates the calving profile for cows at the University of Missouri Forage Systems Research Center in Linneus, MO, over a two year period. This herd maintains a 45-day breeding season, and until the spring of 2004, estrus synchronization and AI were not utilized. Figure 15 illustrates the calving profile of cows that calved during the spring of 2004 as a result of natural service during the 2003 breeding season. Figure 15 also illustrates the calving profile for cows that calved during the spring of 2005 as a result of fixed-time AI performed during the 2004 breeding season (Schafer, 2005; Patterson et al., 2006). This herd has been intensively managed over the years to breed successfully in a 45-day period with natural service. Notice, however, the increased percentage of cows that calved early in the calving period as a result of fixed-time AI performed during the previous year's breeding season. Estrus synchronization at this location in one year resulted in an increase of 7 days postpartum among cows at the start of the breeding period, which translates into an increase in calf age at weaning of seven calf days. These figures (Figures 14, 15) collectively demonstrate that estrus synchronization can be used effectively to influence calving distribution patterns during the subsequent calving period, which in turn impacts the economics of herds at weaning time.



**Figure 14.** Calving distributions combined for 3 of the 4 locations in the study by Schafer, 2005).



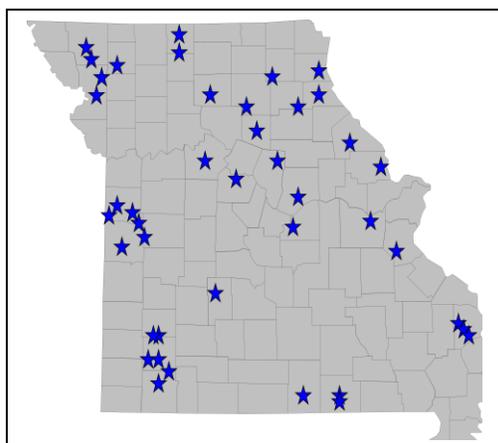
**Figure 15.** Calving profiles for cows at the University of Missouri Forage Systems Research Center in Linneus, MO, over a 2 year period. This herd maintains a 45-day breeding season and until the spring of 2004 estrus synchronization and AI had not been utilized. The figure illustrates the calving profiles of cows that calved during the spring of 2004 as a result of natural service during the 2003 breeding season, and calving profiles for cows that calved during the spring of 2005 as a result of fixed time AI performed during the 2004 breeding season (Schafer, 2005).

Lamb (2015) initiated a case study during the spring of 2008 and reported results through the spring 2013 breeding season, in a cow/calf operation consisting of 300 cows in the Florida panhandle. Prior to the 2008 breeding season the herd was exposed for a 120 day breeding season. The goal was to reduce the length of the breeding season to 70 days within 4 years. Beginning in 2008, all females were subject to the following criteria: 1) replacement heifers were expected to become pregnant during the first 25 days of the breeding season; 2) every cow was exposed to estrus synchronization and fixed-time AI; 3) cows were expected to produce a live calf annually and calve without assistance or were culled; 4) every cow must provide the resources for the genetic potential of the calves and each calf a cow produced must be genetically capable of performing; 5) every cow was expected to maintain body condition; and 6) any cow with an undesirable temperament or disposition was culled. Table 13 highlights the economic impact resulting from improvements in reproductive management involving the shortened breeding season, rigorous culling, and use of fixed-time AI. Note the significant improvement over time that occurred in relation to the mean calving day for the herd, the per dollar increase in calf value, and the overall increase in value of the herd that resulted. As a result of incorporating multiple reproductive management practices, the breeding season was reduced from 120 to 70 days. The net result was a more compact calving season that increased the value of calves by \$169 per calf or an annual increase in calf value for the 300 head operation of \$50,700 per year.

Year	2006	2007	2008	2009	2010	2011	2012	2013
Mean calving day	79.2	80.9	59.2	56.2	53.7	47.2	39.5	38.7
Difference from 2006/2007	0	0	21.7	24.7	27.2	33.7	41.4	42.2
Per calf increase in value	0	0	\$87	\$99	\$109	\$135	\$166	\$169
Herd increase in value	0	0	\$19,100	\$29,700	\$32,700	\$40,500	\$49,800	\$50,700

#### **FIELD DEMONSTRATIONS IN MISSOURI WITH FTAI IN POSTPARTUM BEEF COWS**

Table 14 summarizes results from on-farm field demonstrations conducted in Missouri (Figure 16) involving 73 herds and 7,028 cows. The pregnancy rates shown in Table 14 summarize results from FTAI in postpartum beef cows using the CO-Synch + CIDR protocol with FTAI performed 66 hours after CIDR removal and PG administration. Bear in mind, no heat detection was performed on these farms; cows were inseminated at the predetermined fixed-time without detecting estrus. Pregnancy rates resulting from FTAI averaged 62% for the 73 herds. Interestingly, only 7 herds reported pregnancy rates lower than 50%. Producers in these regions and across Missouri now understand that the technology exists to successfully inseminate postpartum beef cows at predetermined fixed times without the need to detect estrus. Increased profits can be achieved through changes in calving distribution patterns of herds and higher percentages of cows will calve during a more concentrated time frame and earlier in the calving period.



**Figure 16.** Locations of on-farm field demonstrations in Missouri. Cows at these various locations were synchronized using the CO-Synch + CIDR protocol and were inseminated 66 hours after CIDR removal and PG.

**Table 14.** Pregnancy rates resulting from on-farm field demonstrations in Missouri following administration of the CO-Synch + CIDR protocol with fixed-time AI performed 66 hours after PG and CIDR removal.

Item	Herds	Cows inseminated	AI pregnancy rate (mean)	AI pregnancy rate (range)
Fixed-time AI results	73	7028	4327/7028 62%	38-86%*

\*Only 7 of the 73 herds realized pregnancy rates < 50% resulting from fixed-time AI.

### SUMMARY AND CONCLUSIONS

Results presented in this manuscript clearly suggest that available methods of inducing and synchronizing estrus in order to facilitate fixed-time AI in postpartum beef cows create the opportunity to significantly expand the use of artificial insemination in the U.S. cow herd. Collectively, these data point to the fact that the U.S. beef industry finds itself at a unique point in time, unlike any other. Improvements in reproductive technologies enable beef producers to utilize artificial insemination without the need to detect estrus; existing and emerging genomic/genetic technologies enable beef producers to make more rapid strides toward improving the quality of beef they produce; and producers' ability to access and target specific marketing grids enable them to be rewarded for specific quality endpoints. In an editorial authored by Troy Marshall in BEEF magazine (2011), Marshall proposed that as prices and costs increase, traits of efficiency and quality will become bigger drivers of profitability than ever before, and the commodity model of U.S. beef production in all likelihood will no longer be viable. Beef producers in the U.S. have the tools in hand to ensure our country's ranking as the leading global supplier of high quality beef. As we look to the future, the challenge our industry faces is whether these tools will be used to the extent that enables future generations in the U.S. to compete in a global arena, and if so, how effectively.

## REFERENCES

- Abel, J. M., B. E. Bishop, J. M. Thomas, M. R. Ellersieck, S. E. Pooch, M. F. Smith, and D. J. Patterson. 2017. Comparing strategies to synchronize estrus and ovulation prior to fixed-time artificial insemination in primiparous two-year-old beef cows. *Theriogenology*. Volume 87, Number 1: 306-315.
- Ahmad, N., F. N. Schrick, R. L. Butcher, and E. K. Inskeep. 1995. Effect of persistent follicles on early embryonic losses in beef cattle. *Biol. Reprod.* 52:1129-1135.
- Allrich, R. D. 1994. Endocrine and neural control of estrus in dairy cows. *J. Dairy. Sci.* 77:2738-2744.
- Bader, J. F., F. N. Kojima, D. J. Schafer, J. E. Stegner, M. R. Ellersieck, M. F. Smith, and D. J. Patterson. 2005. A comparison of progestin-based protocols to synchronize ovulation and facilitate fixed-time artificial insemination in postpartum beef cows. *J. Anim. Sci.* 83: 136-143.
- Bao, B., and H. A. Garverick. 1998. Expression of steroidogenic enzyme and gonadotropin receptor genes in bovine follicles during ovarian follicular waves: A review. *J. Anim. Sci.* 76:1903-1921.
- Bishop, BE, JM Thomas, JM Abel, SE Pooch, MR Ellersieck, MF Smith, and DJ Patterson. 2016. Split-time artificial insemination in beef cattle: I. Using estrous response to determine optimal time(s) at which to administer GnRH in beef heifers and postpartum cows. *Theriogenology*. Volume 86, Number 4: 1102-1110.
- Bishop, BE, JM Thomas, JM Abel, MR Ellersieck, SE Pooch, MF Smith, and DJ Patterson. 2017. Split-time artificial insemination in beef cattle: III. Comparing fixed-time artificial insemination to split-time artificial insemination with delayed GnRH administration in postpartum cows. *Theriogenology*. Volume 99: 48-52.
- Bremer, V.R., S.M. Damiana, F.A. Ireland, D.B. Faulkner and D.J. Kesler. 2004. Optimizing the interval from PGF to timed Ai in the CO-Synch+CIDR and 7-11 Synch estrus synchronization protocols for postpartum beef cows. *J. Anim. Sci.* 82(Suppl. 2):106.
- Bridges, G. A., L. A. Helser, D. E. Grum, M. L. Mussard, C. L. Gasser, and M. L. Day. 2008. Decreasing the interval between GnRH and PGF<sub>2α</sub> from 7 to 5 days and lengthening proestrus increases timed-AI pregnancy rates in beef cows. *Theriogenology* 69: 843-851.
- Bridges, G. A., M. L. Mussard, L. A. Helser, and M. L. Day. 2009. Comparison of follicular dynamics and hormone concentrations between the 7 d and 5 d CO-Synch + CIDR program in two-year-old beef cows. *J. Anim. Sci.* 87(E-supplement): 464.
- Bridges, G. A., J. K. Ahola, C. Brauner, L. H. Cruppe, J. F. Currin, M. L. Day, P. J. Gunn, J. R. Jaeger, S. L. Lake, G. C. Lamb, H. L. Marquezini, R. K. Peel, A. E. Radunz, J. S. Stevenson, J. C. Whittier, and W. D. Whittier. 2012. Determination of appropriate delivery of PGF<sub>2α</sub> in the 5-day CO-Synch + CIDR protocol in lactating beef cows. *J. Anim. Sci.* 90:4814-4822.
- Brown, L. N., K. G. Odde, D. G. LeFever, M. E. King, and C. J. Neubauer. 1988. Comparison of MGA-PGF<sub>2α</sub> to Syncro-Mate B for estrous synchronization in beef heifers. *Theriogenology* 30:1.
- Burke, J. M., R. L. d la Sota, C. A. Risco, C. R. Staples, E.J. P. Schmitt, and W. W. Thatcher. 1996. Evaluation of timed insemination using a gonadotropin-releasing agonist in lactating dairy cows. *J. Dairy Sci.* 79:1385-1393.

- Busch, D. C., D. J. Wilson, D. J. Schafer, N. R. Leitman, J. K. Haden, M. R. Ellersieck, M. F. Smith, and D. J. Patterson. 2007. Comparison of CIDR-based estrus synchronization protocols prior to fixed-time AI on pregnancy rate in beef heifers. *J. Anim. Sci.* 85:1933-1939.
- Busch, D. C., D. J. Schafer, D. J. Wilson, D. A. Mallory, N. R. Leitman, J. K. Haden, M. R. Ellersieck, M. F. Smith, and D. J. Patterson. 2008. Timing of artificial insemination in postpartum beef cows following administration of the CO-Synch + controlled internal drug release protocol. *J. Anim. Sci.* 86:1519-1525.
- DeJarnette, J. M., M. L. Day, R. B. House, R. A. Wallace, and C. E. Marshall. 2001a. Effect of GnRH pretreatment on reproductive performance of postpartum suckled beef cows following synchronization of estrus using GnRH and PGF<sub>2α</sub>. *J. Anim. Sci.* 79: 1675-1682.
- DeJarnette, J. M., R. A. Wallace, R. B. House, R. R. Salverson, and C. E. Marshall. 2001b. Attenuation of premature estrous behavior in postpartum beef cows synchronized to estrus using GnRH and PGF<sub>2α</sub>. *Theriogenology* 56:493-501.
- DeJarnette, J. M., R. L. Nebel, C. E. Marshall, J. F. Moreno, C. R. McCleary, and R. W. Lenz. 2008. Effect of sex-sorted sperm dosage on conception rates in Holstein heifers and lactating cows. *J. Dairy Sci.* 91:1778-1785.
- Den Daas, J. H., G. DeJonh, L. A. M. Lansbergen, and A. M. Van Wagtendonk-Deleeuw. 1998. The relationship between the number of sperm inseminated and the reproductive efficiency of individual dairy bulls. *J. Dairy Sci.* 81:1714-1723.
- Deutscher, G. H. 2000. Extending interval from seventeen to nineteen days in the melengestrol acetate-prostaglandin estrous synchronization program for heifers. *Prof. Anim. Sci.* 16:164-168.
- 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.
- Dziuk, P. J., and R. A. Bellows. 1983. Management of reproduction in beef cattle, sheep and pigs. *J. Anim. Sci.* 57(Suppl.2), 355.
- Federal Register. March 26, 1997. New animal drugs for use in animal feeds; Melengestrol Acetate. Vol. 62. No.58. pp.14304-14305.
- Fortune, J. E., J. Sirois, and S. M. Quirk. 1988. The growth and differentiation of ovarian follicles during the bovine estrous cycle. *Theriogenology* 29:95-109.
- Garverick, H. A., R. G. Elmore, D. H. Vaillancourt, and A. J. Sharp. 1980. Ovarian response to gonadotropin-releasing hormone in postpartum dairy cows. *Amer. J. Vet. Res.* 41:1582-1585.
- Geary, T. W., J. C. Whittier, E. R. Downing, D. G. LeFever, R. W. Silcox, M. D. Holland, T. M. Nett, and G. D. Niswender. 1998a. Pregnancy rates of postpartum beef cows that were synchronized using Syncro-Mate B or the Ovsynch protocol. *J. Anim. Sci.* 76:1523.
- Geary, T. W., J. C. Whittier, and D. G. LeFever. 1998b. Effect of calf removal on pregnancy rates of cows synchronized with the Ovsynch or CO-Synch protocol. *J. Anim. Sci.* 81(Suppl.1)278.
- Geary, T. W., E. R. Downing, J. E. Bruemmer, and J. C. Whittier. 2000. Ovarian and estrous response of suckled beef cows to the Select Synch estrous synchronization protocol. *Prof. Anim.Sci.* 16:1-5.
- Hansel, W., P. V. Malven, and D. L. Black. 1961. Estrous cycle regulation in the bovine. *J. Anim. Sci.* 20:621-625.

- Hill S. L., D. M. Grieger, K. C. Olson, J. R. Jaeger, C. R. Dahlen, M. R. Crosswhite, N. N. Pereira, S. R. Underdahl, B. W. Neville, J. Ahola, M. C. Fischer, G. E. Seidel, and J. S. Stevenson. 2016a. Gonadotropin-releasing hormone increased pregnancy risk in suckled beef cows not detected in estrus and subjected to a split-time artificial insemination program. *J. Anim. Sci.* 94:3722-3728.
- Hill S. L., D. M. Grieger, K. C. Olson, J. R. Jaeger, C. R. Dahlen, G. A. Bridges, F. Dantas, J. E. Larson, A. M. Muth-Spurlock, J. Ahola, M. C. Fischer, G. A. Perry, E. L. Larimore, T. L. Steckler, W. D. Whittier, J. F. Currin, and J. S. Stevenson. 2016b. Using estrus detection patches to optimally time insemination improved pregnancy risk in suckled beef cows enrolled in a fixed-time artificial insemination program. *J. Anim. Sci.* 94:3703-3710.
- Hollinshead, F. K., L. Gillan, J. K. O'Brien, G. Evans, and W. M. Maxwell. 2003. In vitro and in vivo assessment of functional capacity of flow cytometrically sorted ram spermatozoa after freezing and thawing. *Reprod. Fertil. Dev.* 15:351-359.
- Hughes, H. Something's amiss with profit part 1. *BEEF*. February 1, 2005.
- Imwalle, D. B., D. J. Patterson, K. K. Schillo. 1998. Effects of melengestrol acetate on onset of puberty, follicular growth, and patterns of luteinizing hormone secretion in beef heifers. *Biol. Reprod.* 58:1432-1436.
- Johnson, S. K., and R. Jones. 2004. Cost and comparisons of estrous synchronization systems. In proceedings Applied Reproductive Strategies in Beef Cattle. North Platte, NE. pp103-115.
- Kasimanickam, R., M. L. Day, J. S. Rudolph, J. B. Hall, and W. D. Whittier. 2008. Comparison of one versus two doses of prostaglandin  $F_{2\alpha}$  in a 5-day, progesterone-based synchronization protocol in Angus-cross beef cows. *Theriogenology* 70: 585-585.
- Kojima, F. N., B. E. Salfen, J. F. Bader, W. A. Rieke, M. C. Lucy, M. F. Smith, and D. J. Patterson. 2000. Development of an estrus synchronization protocol for beef cattle with short-term feeding of melengestrol acetate: 7-11 Synch. *J. Anim. Sci.* 78:2186-2191.
- Lamb, G. C. 2015. Impacts of estrous synchronization on cowherd performance. In: Proc. Range Beef Cow Symposium XXIV. Loveland, CO. pp. 81-88.
- Lamb, G. C., D. W. Nix, J. S. Stevenson, and L. R. Corah. 2000. Prolonging the MGA-prostaglandin  $F_{2\alpha}$  interval from 17 to 19 days in an estrus synchronization system for heifers. *Theriogenology* 53:691-698.
- Lamb, G. C., J. S. Stevenson, D. J. Kesler, H. A. Garverick, D. R. Brown, and B. E. Salfen. 2001. Inclusion of an intravaginal progesterone insert plus GnRH and prostaglandin  $F_{2\alpha}$  for ovulation control in postpartum suckled beef cows. *J. Anim. Sci.* 79:2253-2259.
- Lamond, D. R. 1964. Synchronization of ovarian cycles in sheep and cattle. *Anim. Breed. Abstr.* 32:269-285.
- 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 insuckled beef cows for detected estrus and artificial insemination and timed artificial insemination using gonadotropin-releasing hormone, prostaglandin  $F_{2\alpha}$ , and progesterone. *J. Anim. Sci.* 84:332-342.
- Lauderdale, J. W. 1972. Effects of prostaglandin  $F_{2\alpha}$  on pregnancy and estrous cycle of cattle. *J. Anim. Sci.* 35(Suppl. 1):246.
- Lauderdale, J. W., B. E. Seguin, J. N. Stellflug, J. R. Chenault, W. W. Thatcher, C. K. Vincent, and A. F. Loyancano. 1974. Fertility of cattle following PGF $_{2\alpha}$  injection. *J. Anim. Sci.* 38:964-967.

- Leitman, N. R., D. C. Busch, J. F. Bader, D. A. Mallory, D. J. Wilson, M. C. Lucy, M. R. Ellersieck, M. F. Smith, and D. J. Patterson. 2008. Comparison of protocols to synchronize estrus and ovulation in estrous-cycling and prepubertal beef heifers. *J. Anim. Sci.* 86: 1808-1818.
- Leitman, N. R., D. C. Busch, D. J. Wilson, D. A. Mallory, M. R. Ellersieck, M. F. Smith, and D. J. Patterson. 2009. Comparison of controlled internal drug release insert-based protocols to synchronize estrus in prepubertal and estrous-cycling beef heifers. *J. Anim. Sci.* 87: 3976-3982.
- Liehr, R. A., G. B. Marion, and H. H. Olson. 1972. Effects of prostaglandin on cattle estrous cycles. *J. Anim. Sci.* 35(Suppl. 1):247.
- Lopez, H., R. Sartori, and M. C. Wiltbank. 2005. Reproductive hormones and follicular growth during development of one or multiple dominant follicles in cattle. *Biol. Reprod.* 72: 788-795.
- Lucy, M. C., H. J. Billings, W. R. Butler, L. R. Ehnis, M. J. Fields, D. J. Kesler, J. E. Kinder, R. C. Mattos, R. E. Short, W. W. Thatcher, R. P. Wettemann, J. V. Yelich, and H. D. Hafs. 2001. Efficacy of an intravaginal progesterone insert and an injection of PGF<sub>2α</sub> for synchronizing estrus and shortening the interval to pregnancy in postpartum beef cows, periparturient beef heifers, and dairy heifers. *J. Anim. Sci.* 79:982-995.
- Mallory, D. A., J. M. Nash, M. R. Ellersieck, M. F. Smith, and D. J. Patterson. 2011. Comparison of long-term progestin-based protocols to synchronize estrus before fixed-time artificial insemination in beef heifers. *J. Anim. Sci.* 89: 1358-1365.
- Mallory, D. A., S. L. Lock, D.C. Woods, S. E. Pooch, and D. J. Patterson. 2013. Comparison of sex-sorted and conventional semen within a fixed-time artificial insemination protocol for dairy heifers. *J. Dairy. Sci.* 96:854-856.
- Macmillan, K.L., and H.V. Henderson. 1984. Analyses of the variation in the interval of prostaglandin F<sub>2α</sub> to oestrus as a method of studying patterns of follicle development during diestrus in dairy cows. *Anim. Reprod. Sci.* 6:245-254.
- Macmillan, K. L., and J. D. Watson. 1975. Fertility differences between groups of sires relative to the stage of oestrus at the time of insemination. *Anim. Prod.* 21:243-249.
- Markwood, M. G., R. K. Peel, J. K. Ahola, G. E. Seidel, R. N. Funston, S. L. Lake, and J. C. Whittier. 2014. Effect of delaying time AI based on ESTROTECT™ patch status on pregnancy rates of nursing beef cows. *Proc. Am. Soc. Anim. Sci. West Sect*; 65:79-82.
- Marshall, T. 2011. The quality revolution awakens. In: BEEF Cow Calf Weekly. July 8, 2011.
- Martin, N. T., J. M. Thomas, J. M. Nash, D. A. Mallory, M. R. Ellersieck, S. E. Pooch, M. F. Smith, and D. J. Patterson. 2014. Comparison of a 16- versus a 19-day interval between controlled internal drug release removal and prostaglandin F<sub>2α</sub> following a 14-day controlled internal drug release treatment and fixed-time artificial insemination in postpartum beef cows. *J. Anim. Sci.* 92:1757-1765.
- Nash, J. M., D. A. Mallory, M. R. Ellersieck, S. E. Pooch, M. F. Smith, and D. J. Patterson. 2012. Comparison of long- versus short-term CIDR-based protocols to synchronize estrus prior to fixed-time AI in postpartum beef cows. *Anim. Reprod. Sci.* 132(1-2): 11-16.
- Nash, J. M., D. A. Mallory, M. R. Ellersieck, S. E. Pooch, M. F. Smith, and D. J. Patterson. 2013. Comparison of long-term controlled internal drug release-based protocols to synchronize estrus and ovulation in postpartum beef cows. *J. Anim. Sci.* 91: 3168-3176.
- Nellor, J.E., and H.H. Cole. 1956. The hormonal control of estrus and ovulation in the beef heifer. *J. Anim. Sci.* 15:650-661.

- NAHMS. 1998. Part IV. Changes in the U.S. Beef Cow-Calf Industry. 1993-1997. pp. 1. USDA-APHIS Center for Epidemiology and Animal Health, Fort Collins, CO.
- Patterson, D. J., and D. S. Brown. 2013. Rebuilding the U.S. beef herd: Rethinking the way industry develops replacements. In: *Veterinary Clinics of North America: Food Animal Practice*. 29:469-477.
- Patterson, D. J., G. H. Kiracofe, J. S. Stevenson, and L. R. Corah. 1989. Control of the bovine estrous cycle with melengestrol acetate (MGA): A review. *J. Anim. Sci.* 67:1895-1906.
- Patterson, D. J., J. B. Hall, N. W. Bradley, K. K. Schillo, B. L. Woods, and J. M. Kearnan. 1995. Improved synchrony, conception rate, and fecundity in postpartum suckled beef cows fed melengestrol acetate prior to prostaglandin F<sub>2α</sub>. *J. Anim. Sci.* 73:954.
- Patterson, D. J., S. L. Wood, F. N. Kojima, and M. F. Smith. 2000. Current and emerging methods to synchronize estrus with melengestrol acetate. In: 49<sup>th</sup> Annual Beef Cattle Short Course Proceedings "Biotechnologies of Reproductive Biology". Pp. 45-66. University of Florida, Gainesville.
- Patterson, D. J., J. E. Stegner, F. N. Kojima, and M. F. Smith. 2002. MGA<sup>®</sup> Select improves estrus response in postpartum beef cows in situations accompanied with high rates of anestrus. *Proc. West. Sec. Am. Soc. Anim. Sci.* 53:418-420.
- Patterson, D.J., F.N. Kojima, and M.F. Smith. 2003. A review of methods to synchronize estrus in replacement heifers and postpartum beef cows. *J. Anim. Sci.* 81(E. Suppl. 2):E166-E177. Online. Available: <http://www.asas.org/symposia/03esupp2/jas2402.pdf>. Accessed June 19, 2003.
- Patterson, D. J., D. J. Schafer, D. C. Busch, N. R. Leitman, D. J. Wilson, and M. F. Smith. 2006. Review of estrus synchronization systems: MGA. In: *Proceedings Applied Reproductive Strategies in Beef Cattle*. St. Joseph, MO. Pp. 63-103.
- Patterson, D.J., D.C. Busch, N.R. Leitman, D.J. Wilson, D.A. Mallory, and M.F. Smith. 2007. Estrus synchronization protocols for heifers. *Proc Applied Reproductive Strategies in Beef Cattle*, Billings, MT: 63-97.
- Perry, G. A., M. F. Smith, and D. J. Patterson. 2002. Evaluation of a fixed-time artificial insemination protocol for postpartum suckled beef cows. *J. Anim. Sci.* 80:3060-3064.
- Perry, G.A, M.F. Smith, and T.W. Geary. 2004. Ability of intravaginal progesterone inserts and melengestrol acetate to induce estrous cycles in postpartum beef cows. *J. Anim. Sci.* 82:695-704.
- Perry, G.A., M.F. Smith, M.C. Lucy, J.A. Green, T.E. Parks, M.D. MacNeil, A.J. Roberts and T.W. Geary. 2005. Relationship between follicle size at insemination and pregnancy success. *PNAS* 102(14):5268-5273.
- Perry, G.A., B.L. Perry, J.H. Krantz, and J. Rodgers. 2012. Influence of inducing luteal regression prior to a fixed-time AI CIDR protocol in postpartum beef cows on pregnancy success. *J. Anim. Sci.* 90:489-494.
- Perry, G.A. and M.F. Smith. 2015. Management factors that impact the efficiency of applied reproductive strategies. In: *Proceedings, Applied Reproductive Strategies in Beef Cattle*. August 17-18. Davis, CA. pp. 208-232.
- Pohler, K. G., T. W. Geary, J. A. Atkins, G. A. Perry, E. M. Jinks, and M. F. Smith. 2012. Follicular determinants of pregnancy establishment and maintenance. *Cell. Tissue. Res.* 349:649-664.
- Pursley, J. R., M. O. Mee, and M. C. Wiltbank. 1995. Synchronization of ovulation in dairy cows using PGF<sub>2α</sub> and GnRH. *Theriogenology* 44:915-924.

- Pursley, J. R., M. W. Kosorok, and M. C. Wiltbank. 1997a. Reproductive management of lactating dairy cows using synchronization of ovulation. *J. Dairy Sci.* 80:301-306.
- Pursley, J. R., M. C. Wiltbank, J. S. Stevenson, J. S. Ottobre, H. A. Garverick, and L. L. Anderson. 1997b. Pregnancy rates in cows and heifers inseminated at a synchronized ovulation or synchronized estrus. *J. Dairy Sci.* 80:295-300.
- Pursley, J. R., R. W. Silcox, and M. C. Wiltbank. 1998. Effect of time of artificial insemination on pregnancy rates, calving rates, pregnancy loss, and gender ratio after synchronization of ovulation in lactating dairy cows. *J. Dairy Sci.* 81:2139-2144.
- Revah, I., and W. R. Butler. 1996. Prolonged dominance of follicles and reduced viability of bovine oocytes. *J. Reprod. Fertil.* 106:39-47.
- Roche, J. F., E. J. Austin, M. Ryan, M. O'Rourke, M. Mihm, and M. G. Diskin. 1999. Regulation of follicle waves to maximize fertility in cattle. *J. Reprod. Fertil. Suppl.* 54:61-71.
- Rowson, L.E.A., R. Tervit, and A. Brand. 1972. The use of prostaglandin for synchronization of oestrus in cattle. *J. Reprod. Fertil.* 29:145 (Abstr).
- Sá Filho, O. G., D. J. Patterson, and J. L. M. Vasconcelos. 2009. Development of estrous synchronization protocols using melengestrol acetate in *Bos indicus* cattle. *J. Anim. Sci.* 87: 1981-1990.
- Sartori, R., P. M. Fricke, J. C. Ferreira, O. J. Ginther, and M. C. Wiltbank. 2001. Follicular deviation and acquisition of ovulatory capacity in bovine follicles. *Biol. Reprod.* 65:1403-1409.
- Schafer, D. J. 2005. Comparison of progestin based protocols to synchronize estrus and ovulation in beef cows. M.S. Thesis. University of Missouri, Columbia.
- Schafer, D. J., J. F. Bader, J. P. Meyer, J. K. Haden, M. R. Ellersieck, M. C. Lucy, M. F. Smith, and D. J. Patterson. 2007. Comparison of progestin based protocols to synchronize estrus and ovulation before fixed-time artificial insemination in postpartum beef cows. *J. Anim. Sci.* 85:1940-1945.
- Schafer, D.W., J.S. Brinks, and D.G. LeFever. 1990. Increased calf weaning weight and weight via estrus synchronization. Beef Program Report. Colorado State University. pp. 115-124.
- Schmitt, E. J.-P., T. Diaz, M. Drost, and W. W. Thatcher. 1996. Use of a gonadotropin-releasing hormone agonist or human chorionic gonadotropin for timed insemination in cattle. *J. Anim. Sci.* 74:1084-1091.
- Seidel, G. E. Jr. 1995. Reproductive biotechnologies for profitable beef production. Proc. Beef Improvement Federation. Sheridan, WY. Pp. 28-39.
- Seidel Jr., G. E. 2007. Overview of sexing sperm. *Theriogenology* 68:443-446.
- Seidel Jr., G. E. 2011. Profitable uses of sex-sorted semen. In: Proc. Applied Reproductive Strategies in Beef Cattle. Joplin, MO. Pp. 349-353.
- Sirois, J., and J. E. Fortune. 1988. Ovarian follicular dynamics during the estrous cycle in heifers monitored by real-time ultrasonography. *Biol. Reprod.* 39:308-317.
- Stegner, J. E., F. N. Kojima, M. R. Ellersieck, M. C. Lucy, M. F. Smith, and D. J. Patterson. 2004a. A comparison of progestin-based protocols to synchronize estrus in postpartum beef cows. *J. Anim. Sci.* 82:1016-1021.
- Stegner, J. E., J. F. Bader, F. N. Kojima, M. R. Ellersieck, M. F. Smith, and D. J. Patterson. 2004b. Fixed-time artificial insemination of postpartum beef cows at 72 or 80 hours after treatment with the MGA<sup>®</sup> Select protocol. *Theriogenology* 61:1299-1305.

- Stegner, J. E., F. N. Kojima, M. R. Ellersieck, M. C. Lucy, M. F. Smith, and D. J. Patterson. 2004c. Follicular dynamics and steroid profiles in cows during and after treatment with progestin-based protocols for synchronization of estrus. *J. Anim. Sci.* 82:1022-1028.
- Stevenson, J. S., G. C. Lamb, J. A. Cartmill, B. A. Hensley, S. Z. El-Zarkouny, and T. J. Marple. 1999. Synchronizing estrus in replacement beef heifers using GnRH, melengestrol acetate, and PGF<sub>2α</sub>. *J. Anim. Sci.* 77(Suppl. 1):225.
- Stevenson, J. S., K. E. Thompson, W. L. Forbes, G. C. Lamb, D. M. Grieger, and L. R. Corah. 2000. Synchronizing estrus and (or) ovulation in beef cows after combinations of GnRH, norgestomet, and prostaglandin F<sub>2α</sub> with or without timed insemination. *J. Anim. Sci.* 78:1747-1758.
- Stevenson, J. S., G. C. Lamb, S. K. Johnson, M. A. Medina-Britos, D. M. Grieger, K. R. Harmony, J. A. Cartmill, S. Z. El-Zarkouny, C. R. Dahlen, and T. J. Marple 2003. Supplemental norgestomet, progesterone, or melengestrol acetate increases pregnancy rates in suckled beef cows after timed inseminations. *J. Anim. Sci.* 81:571-586.
- Thatcher, W. W., K. L. Macmillan, P. J. Hansen, and M. Drost. 1989. Concepts for regulation of corpus luteum function by the conceptus and ovarian follicles to improve fertility. *Theriogenology* 31: 149-164.
- Thatcher, W. W., M. Drost, J. D. Savio, K. L. Macmillan, K. W. Entwistle, E. J. Schmitt, R. L. De La Sota, and G. R. Morris. 1993. New clinical uses of GnRH and its analogues in cattle. *Anim. Reprod. Sci.* 33:27-49.
- Thimonier, J., D. Chupin, and J. Pelot. 1975. Synchronization of estrus in heifers and cyclic cows with progestogens and prostaglandin analogues alone or in combination. *Ann. Biol. Anim. Biochim. Biophys.* 15:437-449.
- Thomas, J. M., S. L. Lock, S. E. Pooch, M. R. Ellersieck, M. F. Smith, and D. J. Patterson. 2014a. Delayed insemination of non-estrous cows improves pregnancy rates when using sex-sorted semen in timed artificial insemination of suckled beef cows. *J. Anim. Sci.* 92:1745-1750.
- Thomas, J. M., S. E. Pooch, M. R. Ellersieck, M. F. Smith, and D. J. Patterson. 2014b. Delayed insemination of non-estrous heifers and cows when using conventional semen in timed artificial insemination. *J. Anim. Sci.* 92:4189-4197.
- Thomas, J. M., B. E. Bishop, J. M. Abel, M. R. Ellersieck, M. F. Smith, and D. J. Patterson. 2016a. The 9-d CIDR-PG protocol: Incorporation of prostaglandin F<sub>2α</sub> pretreatment into a long-term progestin-based estrus synchronization protocol for postpartum beef cows. *Theriogenology.* 85:1555-1561.
- Thomas, J. M., B. E. Bishop, J. M. Abel, J. W. C. Locke, S. E. Pooch, D. S. Brown, M. F. Smith, and D. J. Patterson. 2016b. Split-time AI: Using estrus detection aids to optimize timed artificial insemination. University of Missouri Extension MP739.
- Thomas, J. M., J.W.C. Locke, B.E. Bishop, J.M. Abel, M.R. Ellersieck, S.E. Pooch, M.F. Smith, and D.J. Patterson. 2017. The 9-d CIDR-PG protocol II: Characterization of endocrine parameters, ovarian dynamics, and pregnancy rates to fixed-time AI following use of long-term CIDR-based estrus synchronization among mature beef cows. *Theriogenology.* 103:185-190.
- Twagiramungu, H., L. A. Guilbault, J. Proulx, and J. J. Dufour. 1992a. Synchronization of estrus and fertility in beef cattle with two injections of Buserelin and prostaglandin. *Theriogenology* 38:1131-1144.

- Twagiramungu, H., L. A. Guilbault, J. Proulx, P. Villeneuve, and J. J. Dufour. 1992b. Influence of an agonist of gonadotropin-releasing hormone (Buserelin) on estrus synchronization and fertility in beef cows. *J. Anim. Sci.* 70:1904-1910.
- Twagiramungu, H., L. A. Guilbault, and J. J. Dufour. 1995. Synchronization of ovarian follicular waves with a gonadotropin-releasing hormone agonist to increase the precision of estrus in cattle: A review. *J. Anim. Sci.* 73:3141-3151.
- Ulberg, L. C., R. E. Christian, and L. E. Casida. 1951. Ovarian response in heifers to progesterone injections. *J. Anim. Sci.* 10:752-759.
- Valdez, K. E., S. P. Cuneo, and A. M. Turzillo. 2005. Regulation of apoptosis in the atresia of dominant bovine follicles of the first follicular wave following ovulation. *Reproduction* 130: 71-81.
- Whittier, W. D., J. F. Currin, H. Schramm, S. Holland, and R. K. Kasimanickam. 2013. Fertility in Angus cross beef cows following 5-day CO-Synch + CIDR or 7-day CO-Synch + CIDR estrus synchronization and timed artificial insemination. *Theriogenology*. 80(9):963-969.
- Wilson, D. J., D. A. Mallory, D. C. Busch, N. R. Leitman, J. K. Haden, D. J. Schafer, M. R. Ellersieck, M. F. Smith, and D. J. Patterson. 2010. Comparison of short-term progestin-based protocols to synchronize estrus and ovulation in postpartum beef cows. *J. Anim. Sci.* 88:2045-2054.
- Wood, S. L., M. C. Lucy, M. F. Smith, and D. J. Patterson. 2001. Improved synchrony of estrus and ovulation with addition of GnRH to a melengestrol acetate-prostaglandin F<sub>2α</sub> estrus synchronization treatment in beef heifers. *J. Anim. Sci.* 79:2210-2216.
- Wood-Follis, S. L., F. N. Kojima, M. C. Lucy, M. F. Smith, and D. J. Patterson. 2004. Estrus synchronization in beef heifers with progestin-based protocols. I. Differences in response based on pubertal status at the initiation of treatment. *Theriogenology* 62:1518-1528.
- Zelinski, M. B., N. A. Hirota, E. J. Keenan, and F. Stormshak. 1980. Influence of exogenous estradiol-17 beta on endometrial progesterone and estrogen receptors during the luteal phase of the ovine estrous cycle. *Biol. Reprod.* 23:743-751.
- Zimbelman, R. G. 1963. Maintenance of pregnancy in heifers with oral progestogens. *J. Anim. Sci.* 22:868.
- Zimbelman, R. G., and L. W. Smith. 1966. Control of ovulation in cattle with melengestrol acetate. I. Effect of dosage and route of administration. *J. Reprod. Fertil. (Suppl.1)*:185.
- Zimbelman, R. G., J. W. Lauderdale, J. H. Sokolowski, and T. G. Schalk. 1970. Safety and pharmacologic evaluations of melengestrol acetate in cattle and other animals. A review. *J.A.V.M.A.* 157:1528-1536.