

ESTRUS SYNCHRONIZATION SYSTEMS: GnRH

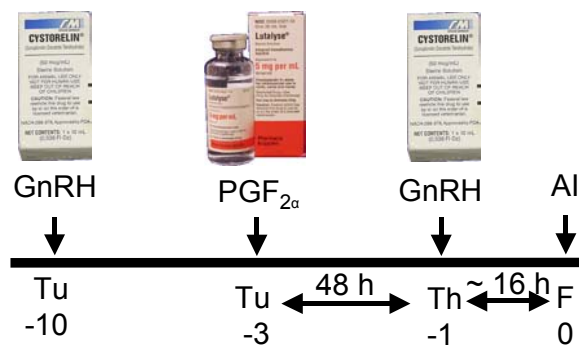
Darrel J. Kesler and Marika Constantaras
Department of Animal Sciences and Veterinary Clinical Medicine
University of Illinois, Urbana, IL

Introduction

Development of methods to manipulate the estrous cycle so that all cows are in estrus during a short, predefined period (synchronized estrus) while maintaining normal fertility has been a difficult goal to achieve, but has resulted in the creation of valuable synchronization protocols that are available to producers today. Although implementation of estrus synchronization and AI will improve the profitability of beef operations, no more than 3 to 5% of all beef operations in the U.S. utilize the technology (Patterson et. al., 2001). The major barriers to utilization of estrus synchronization and AI are time and labor (Kesler, 2003).

During the past 25 years, protocols have been developed that minimize time and labor, and yield excellent pregnancy rates. One of the most important steps to creating the wide variety of effective protocols that are available today began with the understanding of follicular waves and the development of the Ovsynch protocol (illustrated in Figure 1). Ovsynch was originally created for use in dairy cattle, however the basic elements (GnRH followed by PGF₂ α seven days later) have as much value in beef cattle. Three protocols (Select Synch, CO-Synch, and Hybrid Synch) have emerged for use in beef cattle and will be discussed within this manuscript.

Figure 1. Ovsynch protocol



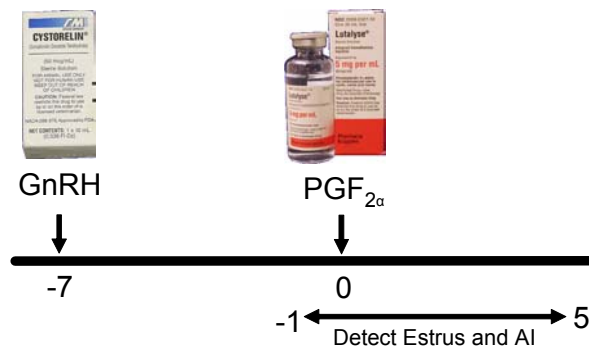
Select Synch

Select Synch, as well as all of the protocols discussed in this review, includes an injection of GnRH followed by PGF₂ α seven days later. The initial injection of GnRH provokes a preovulatory-like LH surge (Pursley et al., 1995). Studies have demonstrated that this single injection of GnRH induces ovulation in most cows, including >80% of late-calving anestrus

cows suckling calves (Thompson et al., 1999). A new follicular wave is then initiated about two days after the GnRH-induced ovulation (Kojima and Patterson, 2003). There are a number of GnRH products available and all seem to have similar efficacy, assuming a full 100 mcg dose is administered. More variable responses, including decreased efficacy, have been reported when cows are administered a half dose of GnRH (John B. Hall, personal communications). Furthermore, 18 g needles that are 1.5 inches long are recommended and GnRH and PGF_{2α} should be injected intramuscularly in the neck.

Seven days after the injection of GnRH cows are administered an injection of PGF_{2α} to induce regression of corpora lutea, if present. Although 25-33% of the estrus-cycling cows will not have corpora lutea and do not need the PGF_{2α}, it is not efficient to attempt to differentiate cows with corpora lutea from those without. Therefore, all cows should receive an injection of PGF_{2α} seven days after the GnRH injection. The protocol is illustrated in Figure 2.

Figure 2. Select Synch protocol

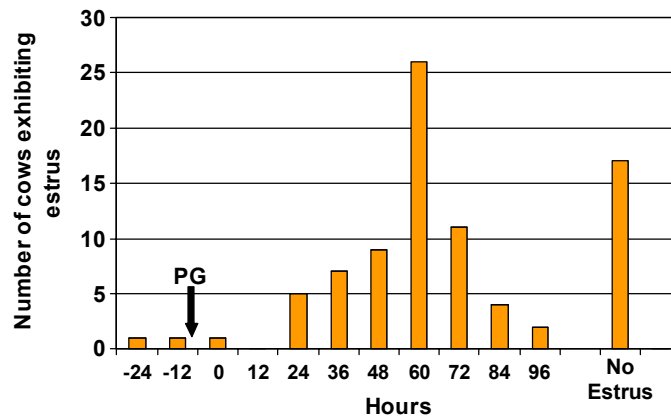


Cows synchronized with the Select Synch protocol are bred based upon the detection of estrus. The majority of cows will exhibit estrus 36 to 72 hours after PGF_{2α} (Stevenson et al., 2000). However, a small percentage will exhibit estrus outside this peak period (see Figure 3), including 8 to 10% that show estrus prior to the injection of PGF_{2α} (Geary et al., 2000). Furthermore, not all cows are detected in estrus—ranging from 7 to 61% in published data. We recommend that estrus detection begin the day before injecting PGF_{2α} followed by 5 days of estrus detection—including the day PGF_{2α} is administered. Although the injection of GnRH may induce the first postpartum ovulation and hasten conception, fertility in cows in poor body condition will still be low (Stevenson et al., 2000; see Table 1).

Body Condition	Pregnancy Rate
4.0 or less	28% (14/50)
4.5	39% (19/49)
5.0 or greater	50% (39/76)

Table 1. Pregnancy rates in suckled beef cows after treatment with Select Synch

Figure 3. Distribution of estrus after Select Synch



The Select Synch procedure was developed for operators who do not object to, or feel more comfortable with, breeding upon the detection of estrus. The Select Synch protocol has been effectively utilized with very encouraging results as reported in Table 2. Overall, pregnancy rates averaged 46% for 1,233 cows. As shown in Table 2, estrus detection rates and pregnancy rates are highly correlated ($r = .96$; $P < .01$). Low responses may be due to compromised estrus detection efficiency, postpartum anestrus, or a combination of both. However, it does illustrate the importance of estrus detection and of using this protocol only when one is fully committed to thorough monitoring of estrus.

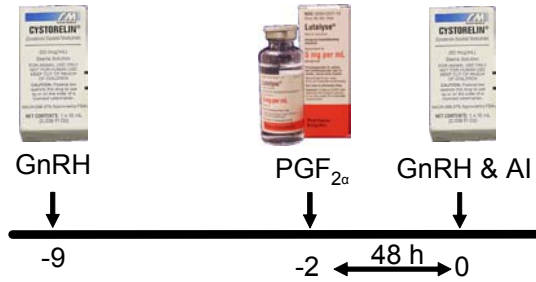
Study	Estrous Response	Pregnancy Rate
Kojima et al., 2000	69% (31/45)	47% (21/45)
DeJarnette et al., 2001a:		
experiment 1	93% (25/27)	70% (19/27)
experiment 2	78% (60/77)	52% (40/77)
Stevenson et al., 2000:		
experiment 1	59% (171/289)	38% (111/289)
experiment 3	63% (116/184)	44% (81/184)
Patterson et al., 2001	67% (353/528)	45% (237/528)
Constantaras et al., 2004	80% (66/83)	65% (54/83)
Range	59% – 93%	38% - 70%
Mean	67% (822/1233)	46% (563/1233)

Table 2. Estrous response rates and pregnancy rates in cows administered the Select Synch protocol

CO-Synch

The CO-Synch protocol utilizes the same strategy as Select Synch; however, it uses a single fixed time AI. The protocol is illustrated in Figure 4. No estrus detection is required with CO-Synch—a major attribute of this protocol. Like Select Synch, cows must be in good body condition as results are compromised in cows in poorer body condition, as illustrated in Table 3 (Lamb et al., 2001).

Figure 4. CO-Synch protocol



Body Condition	Pregnancy Rate
4.5 or less	30% (12/40)
4.5 to 5.0	41% (30/74)
5.5 or greater	59% (19/32)

Table 3. Pregnancy rates in suckled beef cows after treatment with CO-Synch

The CO-Synch protocol has been used in a large number of diverse situations quite successfully. Table 4 is a summary of the available published data where CO-Synch was used. Overall, pregnancy rates have average 44% for 1,562,532 cows. The protocol is quite simple to employ as all injections and timed AI can be done at the same time of the day.

Study	Pregnancy Rates
Stevenson et al., 2000	33% (58/175)
Geary et al., 2001a	49% (57/117)
Geary et al., 2001b	54% (63/117)
Grieger et al., 2001	42% (45/108)
Lamb et al., 2001:	
location 1	52% (26/50)
location 2	54% (50/92)
location 3	38% (36/96)
location 4	53% (26/49)
Perry et al., 2001	47% (53/112)
Larson et al., 2004	43% (234/551)
Constantaras et al., 2004	48% (45/95)
Range	33% - 54%
Mean	44% (693/1562)

Table 4. Pregnancy rates in cows administered the CO-Synch protocol

Some have speculated that short-term calf removal, from the time of PGF_{2α} until AI is completed, may improve pregnancy rates. Geary and co-workers (2001) examined this concept and demonstrated an improvement in one experiment, but not another as illustrated in Table 5. Similar results were observed when short-term calf removal was used with Syncro-Mate B. It is important to note that in order to utilize short-term calf removal one must have excellent holding facilities.

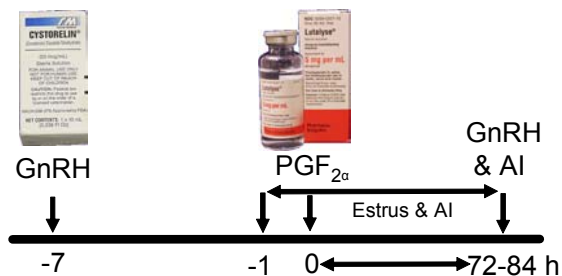
Study	Pregnancy Rates
Geary et al., 2001: with calves calf removal	54% (63/117) 63% (75/119)
Geary et al., 2001: with calves calf removal	49% (57/117) 46% (56/121)

Table 5. Effect of short-term calf removal on pregnancy rates of cows synchronized with CO-Synch

Hybrid Synch

Hybrid Synch, as the name implies, is a blend between Select Synch and CO-Synch. This procedure was created to optimize pregnancy rates in cows administered GnRH-PGF₂α protocol. Because the interval from PGF₂α to estrus is variable, as illustrated in Figure 3, it is impossible to select a single time that all cows have an excellent opportunity to conceive. Therefore, the insemination time for CO-Synch is the single time expected to achieve the highest pregnancy rate—not the optimum time when each individual has the best opportunity to conceive. In order for more cows to have an opportunity to conceive one may breed upon the detection of estrus for a period of time followed by a clean up timed AI—the Hybrid Synch protocol (illustrated in Figure 5). Upon examination of Figure 3, one will note that the highest percentage of cows in this study were in estrus at 60 hours after the PGF₂α injection.

Figure 5. Hybrid Synch protocol



Therefore, the ideal time for clean up timed AI for the majority of the cows is 72 hours. In the Hybrid Synch protocol it is recommended that the clean up timed AI be done at 72 to 84 hours after PGF₂α. This clean up timed AI is only for cows not previously detected in estrus and cows should receive GnRH at insemination. This will improve the likelihood that ovulation will be synchronized with the insemination. Any cows inseminated based on detected estrus **do not** need an injection of GnRH at insemination. Results from published data are summarized in Table 6.

Table 6. Pregnancy rates in cows administered the Hybrid Synch protocol

Study	Estrous Response	Pregnancy Rates
Stevenson et al., 2000	19% (33/177)	34% (60/184)
DeJarnette et al., 2001b: experiment 1	44% (20/45)	44% (20/45)
experiment 2	74% (469/638)	47% (299/632)
Larson et al., 2004		53% (269/513)
DeJarnette et al., 2004: herd A-01	75% (27/36)	51% (18/35)
herd A-02	60% (15/25)	44% (11/25)
herd B-F-01	100% (17/17)	71% (12/17)
herd C-00	75% (9/12)	67% (8/12)
herd C-01	23% (3/13)	23% (3/13)
Range	19 % - 100%	23% - 71%
Mean	62% (593/963)	47% (700/1476)

The results are variable (overall average of 47% for 1,476 cows [data in Table 6]) and do not appear considerably higher than for Select Synch (46%) and CO-Synch (45%); however, it will allow one to maximize the opportunity for obtaining the greatest overall pregnancy rates. Similar to results in Table 2 for Select Synch, the estrous response was correlated ($r = .90$; $P < .01$) to pregnancy rates. Again this suggests that poor estrus detection and/or postpartum anestrus compromised efficacy. Some have even suggested that if the estrus response before the timed AI is poor, following up with the timed AI should be reconsidered.

Select Synch + ReCycleSynch

Because not all cows are inseminated in the Select Synch protocol, cows not detected in estrus and inseminated may be resynchronized for a second breeding. This potentially reduces the time to conception and allows for utilization of AI. This procedure was used on a group of cows by administering CO-Synch beginning six days after the original injection of PGF₂ α to cows that were not observed in estrus and inseminated. Because we started breeding the day before PGF₂ α we had a 16-day breeding period. Pregnancy rate at the end of the Select Synch protocol was 65% (54/83; Constantaras et al., 2004). With the additional cows conceiving to the CO-Synch protocol, the 16 day AI breeding pregnancy rate was 78% (65/83). This is only a slight increase in drug cost as only the cows that were not inseminated after Select Synch were administered CO-Synch; however, there is a significant increase in time and labor.

Heifers

Early studies concluded that GnRH-based protocols with timed AI (Ovsynch and CO-Synch) should not be used in heifers. For example, Martinez et al. (2002) reported pregnancy rates of 39% in heifers synchronized with CO-Synch. This compares to a 68% pregnancy rate in heifers synchronized with a CIDR-based system in the same study (Martinez et al., 2002) and an average 56% pregnancy rate for heifers synchronized with an MGA-based system (14 days of MGA followed by PGF₂ α 19 days after the last day of MGA feeding; Kesler, 2003) in other

studies. More recently, Select Synch has been successfully used in heifers with very good fertility. Lamb et al. (2004) conducted a multi-herd study: heifers were administered Select Synch, two injections of PGF₂α, or the MGA-based system. A greater percentage of MGA treated heifers (83%) were detected in estrus during the target-breeding week than for Select Synch and PGF₂α treated heifers (74% and 75%, respectively). Most of the heifers displayed estrus between 24 and 72 hours. The peak period for Select Synch treated heifers was between 24 and 48 hours after PGF₂α, whereas the peak period for the MGA treated heifers was between 48 and 72 hours. Conception rates ranged from 63 to 68% and pregnancy rates ranged from 47% to 56% and were not different. Funston et al. (2004) also conducted a multi-herd study. They similarly demonstrated that the MGA-based protocol was more effective in synchronizing estrus; however, conception rates and overall AI pregnancy rates for the MGA-based protocol and Select Synch were similar. Combined, these data suggest that Select Synch will effectively synchronize estrus in heifers; however, attempting to time AI is not recommended. Since it is necessary for heifers to be estrus-cycling for Select Synch to be effective, one should assess the reproductive status of the heifers before using Select Synch. This can be accomplished by conducting Reproductive Tract Scores as previously described (Kesler, 2003).

Follicular Dynamics

Research to further understand and/or improve the efficacy of these protocols continues. Follicular dynamics are of particular interest. The use of GnRH at the time of insemination results in a wide range of follicle sizes being ovulated (Perry et al., 2003). Lamb et al. (2001) demonstrated that pregnancy rates increased as follicular size at the time of second GnRH injection (for the CO-Synch protocol) increased to 16.0 to 17.9 mm and then dropped. Furthermore, Mussard et al. (2003) demonstrated that when embryos of similar quality were transferred into cows induced to ovulate small (< 12 mm) or large (> 12 mm) follicles, pregnancy rates were significantly higher in cows that ovulated with large follicles. Therefore, the goal in a timed AI protocol is to administer the second GnRH injection at a time when cows have large follicles, yet before spontaneous ovulation—a difficult goal to achieve.

Estrogens

It is important to point out that some scientists have reported that the use of estrogen—estradiol and estradiol benzoate—may improve synchronization efficacy; however, extensive multi-location studies do not exist to support its use. The consensus of many scientists, including those in the Bovine Reproductive Task Force, is that estradiol use should be suspended for several reasons. First, estradiol and estradiol benzoate are not approved by FDA for this use. Hence, it is not an extra-label use—it is illegal to use estradiol or estradiol benzoate to synchronize estrus and ovulation. The only estrogen approved for use in cattle is estradiol cypionate (ECP[®]). Studies using ECP[®] in beef cattle have been conducted and a protocol (Heat Synch) has been used in dairy cows. The availability of ECP[®] is limited—the last batch has been manufactured. Therefore, protocols discussed in this review are recommended unless a protocol utilizing ECP[®] is in place, but be prepared to shift to another protocol. This recommendation is also based upon a study that reported a higher incidence of invasive breast cancers in women administered a postmenopausal estrogen/progestin product (Women's Health Initiative, 2002). Estrogens will certainly cause breast cancers to proliferate; however, is it a cause of breast

cancer? The Women’s Health Initiative study convinced the public, including a high percentage of physicians, that estrogens cause breast cancer. A smaller arm of the Women’s Health Initiative (2002)—that did not receive significant publicity—was the study where estrogen alone was used in women with hysterectomies. In this study, there was no evidence that estrogen caused cancer (Nelson et al., 2002). However, there is considerable public concern and we do not need to further concern the public with the safety of the product beef producers provide.

Efficacy of Different GnRH Products

The efficacy of the specific GnRH product used with the Select Synch, CO-Synch, and Hybrid Synch protocols has been debated. Much of the discussion was caused by a single study published by Martinez et al., (2003) where some differences were noted (Table 7 and 8) however, another study showed no differences (Cline, 2002). GnRH is a decapeptide—a linear chain of ten amino acids—that is identical for all products. The base for Cystorelin[®], Fertagyl[®], and Ovacyst[™] is diacetate tetrahydrate. Therefore, Cystorelin[®], Fertagyl[®], and Ovacyst[™] are chemically identical. Factrel[®] has an HCl base; however, this variation should not alter bioactivity. Summaries comparing the products are presented in the following two tables (Tables 7 and 8).

Table 7. GnRH products used for synchronization.

Study	Products Used	Results
Martinez et al. 2003—expt. 1 (8 cows)	Cystorelin [®] and Fertagyl [®]	In regards to LH responses there was “. . . no effect of treatment (P=0.13). . .”
Cline 2002 (9-10 cows per group)	Cystorelin [®] and Factrel [®]	“No differences (P=0.55) were detected . . . for area beneath the LH curve.”
Martinez et al.2003—expt. 2 (10 cows per group)	Cystorelin [®] , Fertagyl [®] , and Factrel [®]	“There was . . . a tendency for an effect of treatment (P=.08). . . In particular, peak LH concentrations were higher (P<.01) in the Cystorelin [®] group . . .”
Cline 2002 (9-10 cows per group)	Cystorelin [®] and Factrel [®]	“Maximum concentrations of LH after GnRH administration did not differ among treatments (P=0.62).”
Martinez et al. 2003 —expt. 2 (cows ^a)	Cystorelin [®] , Factrel [®] , and Fertagyl [®]	“Mean day of emergence of the next follicle wave did not differ (P=0.35) among groups.” “Ovulatory rate tended to be higher (P=0.1) in the Cystorelin [®] group . . .”

^aThere were 19, 19, and 7 cows for Cystorelin[®], Fertagyl[®], and Factrel[®] treatments, respectively, for the emergence of the next follicle wave evaluation. There were 18, 11, and 4 cows for Cystorelin[®], Fertagyl[®], and Factrel[®] treatments, respectively, for the ovulatory rate evaluation.

Table 8. GnRH products used for synchronization.

Study	Cystorelin[®]	Factrel[®]	Fertagyl[®]
Martinez et al. 2003 (heifers)			
Peak LH Concentration (ng/mL)	7.6 ^a	6.7 ^{a,b}	6.0 ^b
Wave Emergence (d)	2.0 ^a	2.1 ^a	2.2 ^a
Cline 2002 (cows)			
Wave Emergence (d)	2.0	2.6 ^c	
Ovulatory Follicle Size (mm)	13.2	12.3 ^d	
Day of Ovulation	2.2	2.3 ^e	

^{a,b}Values within rows with different superscripts differ ($P < .05$)

^cWithin row $P = .30$

^dWithin row $P = .59$

^eWithin row $P = .83$

In summary, studies agree that all products evoke a new follicular wave and ovulation, the critical factors to successful synchronization. Although some variations do exist, it is difficult to explain why. Before claiming that there are differences, however, more studies need to be conducted. One must remember, the dose was selected based on the treatment of cystic ovarian disease—the clinical claim for GnRH products. This raises a previously mentioned point. One should use a full dose of GnRH as more variable responses, including decreased efficacy, have been reported when cows are administered a half dose of GnRH (John B. Hall, personal communications). Although all dominant follicles (≥ 10 mm) have the ability to ovulate in response to a GnRH-induced LH surge, Sartori et al. (2001) demonstrated that a larger dose of LH was required to induce ovulation of a 10 mm follicle compared to larger follicles. Certainly, this subject needs further study.

Implications

The purpose of this article is to review the GnRH-based estrus synchronization protocols. A summary is provided in Table 9. It was not the objective to review progestin-based synchronization protocols within this article. A summary of progestin-based systems can be found elsewhere. Although the progestin-based systems may have higher pregnancy rates in some situations, the GnRH-based systems without progestins have value. In fact, a supermarket of estrus synchronization protocols for producers with different needs exists today. Three of the protocols within this estrus synchronization supermarket are Select Synch, CO-Synch, and Hybrid Synch. These are systems minimizing drug costs compared to some others; however, cows must be in good body condition and postpartum anestrus may compromise efficacy.

The protocols described herein do not utilize a progestin (MGA or CIDR). Progestins have been demonstrated to improve efficacy of synchronization and pregnancy rates. However, this is not true in all cases as demonstrated by Lamb and coworkers (2001). In that study cows were synchronized with CO-Synch with or without the CIDR. Cows were divided into four categories: estrus-cycling with a corpus luteum (CL) at $PGF_{2\alpha}$ treatment; estrus-cycling without a CL at $PGF_{2\alpha}$; previously anestrus, yet a CL developed in response to the first injection of GnRH; and anestrus with no luteal development after the first GnRH injection. Pregnancy rates are summarized in Table 10.

Table 9. GnRH/PGF₂α-based estrus synchronization protocols used in beef cows

Protocol	Description
Select Synch	<ul style="list-style-type: none">• The duration of the protocol is only one week; however, breeding should begin six days after initiating the protocol.• It requires minimal drug cost; however, considerable time is required for detection of estrus.• With emphasis on thorough estrus detection, one can obtain excellent pregnancy rates if cows are in good body condition.• AI pregnancy rates may be improved if cows not detected in estrus are subsequently administered CO-Synch.
CO-Synch	<ul style="list-style-type: none">• The duration of this system is nine days.• Because this is a timed AI protocol and all cows are inseminated 48 hours after the injection of PGF₂α, it does not require estrus detection.• At the time of AI, cows are also administered an injection of GnRH which increases the drug cost; however, time and labor are minimized.
Hybrid Synch	<ul style="list-style-type: none">• This is a blend of Select Synch and CO-Synch protocols and maximizes the opportunity for obtaining the greatest overall pregnancy rates and minimizes the risk of unacceptable pregnancy rates..• Cows are bred upon the detection of estrus for the first 72-84 hours. Then any cow not detected in estrus is administered GnRH and inseminated.

The CIDR improved pregnancy rates in three of the four reproduction status categories. When working with a cow herd with a significant percentage of anestrous cows, the protocols described in this article are not highly recommended. In estrus-cycling cows, use of the CIDR only improved pregnancy rates in the cows in which luteolysis occurred before PGF₂α treatment. In this experiment only 28% of the cycling cows were in this category. It is predicted that this improvement was realized only because estrus was delayed in these cows by the CIDR which allowed many to become pregnant to the timed AI. Therefore, it would be unlikely for one to realize an improvement with the CIDR in the Select Synch or Hybrid Synch protocols in cycling cows if estrus detection is good and may not be realized when using CO-Synch. For example, Gasser et al. (2003) synchronized four herds of cyclic beef cows with the CO-Synch protocol either alone or with the CIDR. In two herds pregnancy rates were higher when the CIDR was included. In the other two herds, however, pregnancy rates were lower when the CIDR was included. Overall, pregnancy rates for CO-Synch alone or with the CIDR had identical pregnancy rates (53% when the four herds were averaged). The bottom line is that that the CO-Synch protocol is very effective; however the number in estrus before PGF₂α impacts its effectiveness. Theoretically Hybrid Synch can optimize pregnancy rates by catching all in estrus early and in the end inseminating any cows not detected in heat at the cleanup timed AI. Although evidence of this was not reported in this article, Hybrid Synch will likely prevent extremely low pregnancy rates. These procedures, however, are most effective in cows exhibiting estrus cycles. In a cow herd with a high percentage of anestrous cows, a progestin-

based protocol should be considered. Otherwise, the Select Synch, CO-Synch, and Hybrid Synch protocols are less expensive and yield excellent results.

Table 10. Pregnancy rates in cows synchronized with CO-Synch or CO-Synch + CIDR.

Reproductive Status	CO-Synch	CO-Synch + CIDR	Percentage Point Improvement
Cycling w/ CL at PGF _{2α}	58% (65/112)	58% (71/122)	0
Cycling w/out CL at PGF _{2α}	43% (26/60)	53% (17/32)	10
Anestrus w/CL after GnRH	40% (12/30)	53% (17/32)	13
Anestrus w/out CL after GnRH	38% (10/26)	66% (19/29)	28

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