ESTRUS SYNCHRONIZATION SYSTEMS: GnRH

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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; however, a number of valuable synchronization protocols have been created and 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 (GnRH, PGF seven days later, GnRH 48 hours post-PGF, and AI 16 hours after the second injection of GnRH). Ovsynch was originally created for use in dairy cattle, however the basic elements (GnRH followed by $PGF_2\alpha$ seven days later) have as much value in beef cattle. Three protocols (Select Synch, CO-Synch, and Select Synch + Timed AI) have emerged for use in beef cattle and will be discussed within this manuscript.

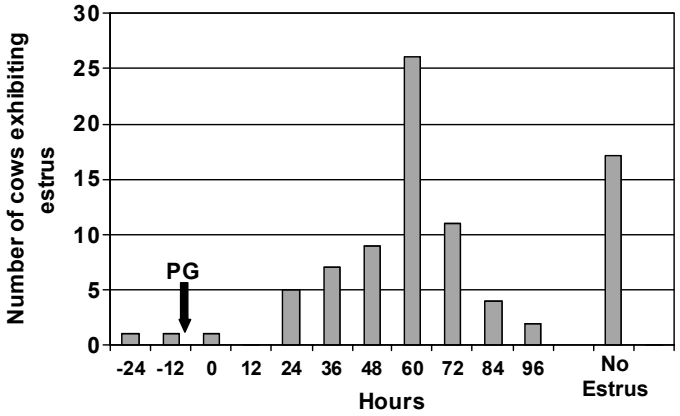
Select Synch

Select Synch, as well as all of the protocols discussed in this review, includes an injection of GnRH followed by $PGF_2\alpha$ 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 anestrous 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 $PGF_2\alpha$ should be injected intramuscularly in the neck. Also, any partially used bottles of GnRH should not be stored long-term as chemical integrity may be compromised.

Seven days after the injection of GnRH cows are administered an injection of $PGF_2\alpha$ 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 $PGF_2\alpha$, it is not efficient to attempt to differentiate cows with corpora lutea from those without corpora lutea. Therefore, all cows should receive an injection of $PGF_2\alpha$ seven days after the GnRH injection.

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 $PGF_2\alpha$ (Stevenson et. al., 2000). However, a small percentage will exhibit estrus outside this peak period (see Figure 1), including 8 to 10% that show estrus prior to the injection of $PGF_2\alpha$ (Geary et al., 2000).

Figure 1
Estrus Distribution with Select Synch



Furthermore, not all cows are detected in estrus—ranging from 7 to 61% in the published data. We recommend that estrus detection begin the day before injecting $PGF_2\alpha$ followed by up to 7 days of estrus detection—including the day $PGF_2\alpha$ 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).

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

Body Condition	Select Synch
4.0 or less	28%
4.5	39%
5.0 or greater	50%

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. 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.

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

Study	Estrus Response	Pregnancy Rate
Kojima et al., 2000	69%	47%
DeJarnette et al., 2001a:		
experiment 1	93%	70%
experiment 2	78%	52%
Stevenson et al., 2000:		
experiment 1	59%	38%
experiment 3	63%	44%
Patterson et al., 2001	67%	45%
Constantaras et al., 2004	80%	65%

CO-Synch

The CO-Synch protocol utilizes the same strategy as Select Synch; however, it uses a single fixed time AI. 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).

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

Body Condition	Select Synch
4.5 or less	30%
4.5 to 5.0	41%
5.5 or greater	59%

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 47%. The protocol is quite simple to employ as all injections and timed AI can be done at the same time of the day. However, details must be followed closely. In the study by Larson et al. (2006) cows were bred at 54 hours after the injection of $PGF_2\alpha$, by design in this case, and pregnancy rates were compromised.

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

Study	**Pregnancy Rates**
Geary and Whittier, 1998:	
location 1	49%
location 2	52%
location 3	46%
Stevenson et al., 2000	33%
Geary et al., 2001	49%
Geary et al., 2001	54%
Stevenson et al., 2003:	
experiment 1	61%
experiment 2	31%
Lamb et al., 2001:	
location 1	52%
location 2	54%
location 3	38%
location 4	53%
Perry et al., 2002	47%
Larson et al., 2004	43%
Constantaras et al., 2004	48%

Some have speculated that short-term calf removal, from the time of $PGF_2\alpha$ 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 facilities. Another advantage of short-term calf removal is that processing of cows is simplified and calf injury is eliminated.

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

Study	Pregnancy Rates
Geary et al., 2001:	
with calves	54%
calf removal	63%
Geary et al., 2001:	
with calves	49%
calf removal	46%

Select Synch & Timed AI

Select Synch & Timed AI is a blend between Select Synch and CO-Synch. This procedure was created to optimize pregnancy rates in cows administered GnRH-$PGF_2\alpha$ protocol. Because

the interval from $PGF_2\alpha$ to estrus is variable, as illustrated in Figure 1, 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 Select Synch & Timed AI protocol. Upon examination of Figure 1, one will note that the highest percentage of cows in this study were in estrus at 60 hours after the $PGF_2\alpha$ injection.

Therefore, the ideal time for clean up timed AI for the majority of the cows is around 72 hours. In the Select Synch & Timed AI protocol it is recommended that the clean up timed AI be done at 72 to 84 hours after $PGF_2\alpha$. This clean up timed AI is only for cows not previously detected in estrus. Furthermore, cows detected in estrus do not need an injection of GnRH at insemination. However, cows at the clean up timed AI should be concurrently administered an injection of GnRH. This will improve the likelihood that ovulation will be synchronized with the insemination. Results from published data are summarized in Table 6.

Table 6. Pregnancy rates in cows administered the Select Synch & Timed AI protocol

Study	Estrus Response	Pregnancy Rates
Stevenson et al., 2000	19%	34%
DeJarnette et al., 2001b:		
experiment 1	44%	44%
experiment 2	74%	47%
Larson et al., 2004		53%
DeJarnette et al., 2004:		
herd A-01	75%	51%
herd A-02	60%	44%
herd B-F-01	100%	71%
herd C-00	75%	67%
herd C-01	23%	23%

The results are variable (overall average of 48% [data in Table 6]) and don't appear considerably higher than for Select Synch (overall average of 52% [data in Table 2]) and CO-Synch (overall average of 47% [data in Table 4]); 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 estrus 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 $PGF_2\alpha$ to cows that were not observed in estrus and inseminated. Because we started breeding the day before $PGF_2\alpha$ we had a 16-day breeding period. Pregnancy rate at the end of the Select Synch protocol was 65% (Constantaras et al., 2006). With the additional cows conceiving to the CO-Synch protocol, the 16 day AI breeding pregnancy rate was 78%. 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.

Bos Indicus

Data discussed to this point has been on European cattle (*Bos taurus*). *Bos taurus* are cattle with the most data; however, in the southern part of the U.S. there are many *Bos indicus* cattle or cattle with *Bos indicus* genetics. The limited *Bos indicus* data are summarized in Table 7.

Study	**Protocol**	**Pregnancy Rates (%)**
Ahuja et al., 2005	Select Synch	0%
Ahuja et al., 2005	CO-Synch	28%
Lemaster et al., 2001	Select Synch	21%
Lemaster et al., 2001	CO-Synch	31%
Lemaster et al., 2001	Select Synch & Timed AI	36%

Because of the poor results (averages of 11%, 30%, and 36% for Select Synch, CO-Synch, and Select Synch & Timed AI, respectively) many researchers have gone to using estrogen rather than GnRH in the synchronization protocols. The use of estrogen will be discussed later. The published data, however, does demonstrate that Select Synch, CO-Synch, and Select Synch & and Timed AI are somewhat efficacious in *Bos indicus* cattle; albeit, lower than when used in B*os taurus* cattle. One factor that will compromise efficacy is the environmental temperature. The *Bos indicus* cattle are in areas with elevated temperatures. Another factor that is often mentioned in many of the *Bos indicus* studies is body condition. These cows often have poor body condition and as demonstrated in the *Bos taurus* cattle body condition will compromise efficacy. Clearly, more research is needed.

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 $PGF_2\alpha$ 19 days after the last day of MGA feeding; Kesler, 2003) in other studies. Select Synch has been successfully used in heifers with good fertility. Lamb et al. (2004) conducted a multi-herd study: heifers were administered Select Synch, two injections of $PGF_2\alpha$, or the MGA-based system. A greater percentage of MGA treated heifers (83%) were detected in estrus during the targeted-breeding week than for Select Synch and $PGF_2\alpha$ 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 $PGF_2\alpha$, 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 inseminate at a predetermined time is not recommended at this time.

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. More recently Perry et al. (2005) demonstrated that GnRH-induced ovulation of follicles 11 mm in diameter or smaller resulted in decreased pregnancy rates and increased late embryonic mortality. This decrease in fertility was associated with lower circulating concentrations of estradiol on the day of insemination, a decreased rate of increase in progesterone after insemination, and, ultimately, decreased circulating concentrations of progesterone. 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. GnRH-induced ovulation of follicles that are physiologically immature, however, has a negative impact on pregnancy rates and late embryonic/fetal survival.

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. Estrogen administration via anabolic implants have been demonstrated to be safe by the FDA. Yet, in 2002 the Women's Health Initiative reported that post-menopausal estrogen therapy increased the incidence of breast cancer. However, this past year, after more thorough review of their data they greatly reduced their warning. This was partially due to the data that demonstrated that estradiol only therapy to post-menopausal women had no increase in breast cancer whatsoever (Nelson et al., 2002). However, there is still considerable public concern and we do not need to further concern the public with the safety of the product beef producers provide. Besides, 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.

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 discussed. Much of the discussion was caused by a study published by Martinez et al., (2003). Martinez et al. (2003) reported that Cystorelin® provoked a greater LH surge than Fertagyl® and Factrel®. Similarly, Cystorelin® induced a higher ovulation rate; however, all products synchronized follicular wave emergence. GnRH is a decapeptide—a linear chain of ten amino acids. The base for Cystorelin®—and Fertagyl® (and Ovacyst™ another GnRH product not included in the Martinez study)—is diacetate, tetrahydrate. Therefore, Cystorelin®, Fertagyl®, and Ovacyst™ are chemically identical. Factrel® has a HCl base which should not alter bioactivity. If the GnRH products are chemically identical, then why did Martinez et al. (2003) observe differences? Being quite familiar with pharmaceutical manufacturing I realize that companies are permitted to include a wide range of active compound in the product. It is unknown if the company manufactures at the low or high end of this range. Hence, the results of Martinez et al. (2003) may only be a difference in active GnRH within the product. 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, has 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 succinct summary is provided in the following table (Table 8).

Although Ovsynch, that utilizes the GnRH-PGF program, was developed for dairy cows and is less attractive for use in beef operations, it has been used successfully (Geary et al., 1998, Lamb et al., 2001). In three studies where it was used pregnancy rates were 51 to 55%. In one operation where Ovsynch was used for five years the owner/operator estimates that 63% of his calves were AI calves as a result of Ovsynch synchronization (Sutphin, 2005). Although his records indicate that there was an increase of $14 per pregnancy ($41/AI pregnancy vs. $27/natural service pregnancy) his records also indicate that there was a reduced death loss with AI (3.5 % vs. 5.5%), more resistance to pneumonia and scours, and less delivery assistance was required (1.3% vs. 2.9%). Overall, his records suggest that the operation realized $145 more profit from AI calves (from AI cows) if they were retained to harvest.

Other scientists are summarizing results utilizing progestins (MGA- and CIDR-based systems) and can be found elsewhere in these proceedings. 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 Select Synch and Timed AI. These are systems

minimizing drug costs compared to some others; however, cows must be in good body condition and postpartum anestrus many compromise efficacy as illustrated in the following table (cows were synchronized with CO-Synch; Table 9).

Table 8. GnRH/$PGF_2\alpha$-based estrus synchronization protocols used in beef cows

Protocol	Description
Select Synch	• The duration of the protocol is only one week; however, breeding should begin six days after initiating the protocol because a percentage of cows exhibit estrus before the injection of $PGF_2\alpha$. • This protocol requires minimal drug cost; however, considerable time is required for detection of estrus. • In order for this protocol to be successful, estrus detection must be emphasized. With emphasis on 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	• 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 $PGF_2\alpha$, it does not require the time and labor associated with detecting estrus. • At the time of AI, cows are also administered an injection of GnRH which increases the drug cost as compared to Select Synch; however, time and labor are minimized.
Select Synch & Timed AI	• This is a blend of Select Synch and CO-Synch protocols and maximizes the opportunity for obtaining the greatest overall 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. Drug costs are reduced as compared to CO-Synch as cows detected in estrus are not administered GnRH at AI. However, labor costs are increased as compared to CO-Synch.

Table 9. Days postpartum and pregnancy rates for CO-Synch synchronized cows.

Days Postpartum	Pregnancy Rate
30-49	29%
50-69	55%
70-89	60%
>90	79%

There isn't one protocol that fits every situation. These protocols are cost-effective and quite efficacious in cows that are in good body condition and have a significant number of days since calving when the synchronization protocols are implemented.

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