

Synchronizing with GnRH

by JACK WHITTIER & TOM GEARY

What is GnRH and how does it work?

A short lesson in endocrinology may help answer this question. *GnRH* is the abbreviation for gonadotropin-releasing hormone, a natural hormone that is released from the hypothalamus in the brain. GnRH causes the release of two other hormones known as gonadotropins from the pituitary gland.

Follicle-stimulating hormone (FSH) and luteinizing hormone (LH) are the gonadotropins that cause follicular growth and ovulation on the ovary. During the normal estrous cycle, ovarian hormones control the release of GnRH, and thus LH and FSH. During estrus, large amounts of GnRH are secreted, which stimulates the release of a high level of LH — termed an LH surge. It is the LH surge that directly causes ovulation of a follicle. The LH surge also initiates development of a new corpus luteum (CL) on the ovary.

In 1994 and 1995, experiments were published from the University of Wisconsin (Pursley et al., 1995) and from Canada (Twagiramungu et al., 1994a), among other locations, that investigated synchronizing ovulation in dairy cows

using GnRH. The objective of the research was to gain control of the reproductive cycle of cows using a sequence of hormone injections to set up the proper events for a fertile ovulation at a predetermined time. The system was named Ovsynch because it was designed to synchronize the ovulation of all cows in the program. Over the last few years, this approach has been investigated in beef cows at Colorado State University (CSU), as well as at other universities.

Can you clarify the terminology used in describing GnRH systems?

There are three primary protocols using GnRH that are being investigated and practiced in the beef and dairy industries today (see Fig. 1).

The first protocol shown in Fig. 1 is the Ovsynch system developed in dairy cows. Because it requires working the cows four times, it is not currently being used widely in the beef industry. To simplify and reduce the number of workings, we compared inseminating cows at the time of the second GnRH injection (CO-Synch) with inseminating 24 hours after the second GnRH injection (Ovsynch). Our data showed no difference in preg-

nancy rates between the two treatments (54% vs. 58% for CO-Synch vs. Ovsynch, respectively; Geary and Whittier, 1999).

The Select Synch system does not use the second injection of GnRH; rather it incorporates detection of estrus and insemination of cows only after an observed estrus. With this system, the cost of the second injection is eliminated, but the time required for estrus detection is greater. Our approach at CSU has been to focus most of our effort on developing a system that gives high results while using timed insemination.

What do you mean by synchronizing ovulation vs. synchronizing estrus?

Ovulation and estrus are two separate events. During the normal reproductive cycle of a cow, the two events usually coincide, with ovulation occurring approximately 30 hours after the onset of behavioral estrus. The characteristic riding activity of behavioral estrus allows cattlemen to identify when a cow is preparing to ovulate so that artificial insemination (AI) can be done at the proper time to enable pregnancy. Behavioral estrus is also an important cue for bulls during natural service and represents the period of time when the cow will stand for mating.

Using the sequence of injections of GnRH-prostaglandin (PGF)-GnRH in Ovsynch and CO-Synch, we mimic the events that result in ovulation. If we are successful, there is no need to watch for behavioral estrus because we can rely on the injection sequence to cause an ovulation at a predicted, synchronized time. In fact, among many cows in the Ovsynch or CO-Synch systems, the second GnRH injection pre-empts the onset of behavioral estrus; the cows do not show signs of heat even though they have a fertile ovulation.

Fig. 1: Time-line diagram of three frequently used synchronization systems that use gonadotropin-releasing hormone (GnRH) and prostaglandin (PGF)

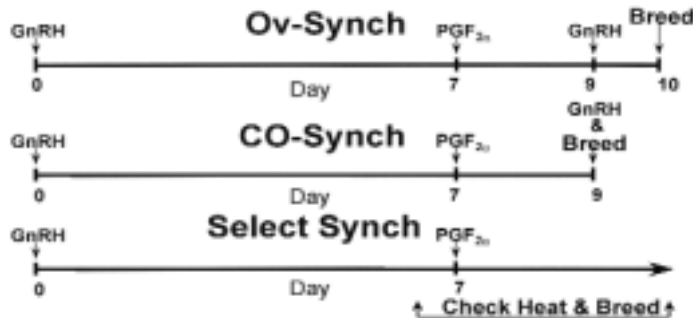
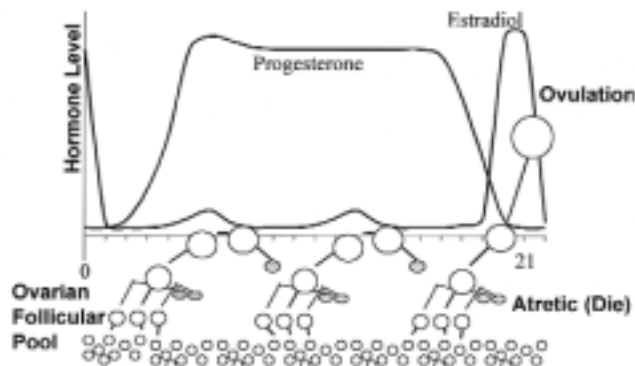


Fig. 2: Development of follicular waves during the 21-day estrous cycle of cattle. Estrogen (E2) levels peak at approximately 212 days, and progesterone (P4) levels are elevated from Day 5 to Day 17



How are the normal events during the estrous cycle simulated with GnRH synchronization systems?

It is important to understand something about development of follicles during the estrous cycle to explain how the GnRH systems work. Ultrasound technology has developed extensively in the past 5-10 years, allowing reproductive physiologists to gain insight into the workings of the cow's estrous cycle. One of the benefits of this understanding is a clearer picture of ovulatory follicle development.

Ovarian follicles grow and regress during the 21-day estrous cycle. The pattern of growth has been described as wave-like (see Fig. 2, left). At the beginning of each

wave of follicle growth, a group of follicles of similar size are present on the ovary and begin to increase in size as the wave proceeds through development, recruitment and selection phases. During each wave, most follicles die one by one until only one growing follicle remains. This follicle, called the dominant follicle, persists for a period of time.

If the proper hormonal signals are sent, the dominant follicle progresses through a final maturing step and ultimately ovulates. The stimulus to ovulate occurs when progesterone levels are low. If the hormonal system does not deliver these signals at the proper time, the follicle regresses (becomes atretic) and does not ovulate. At the same time, however, a new follicular wave is signaled to begin, and subsequently, a new dominant follicle develops.

The primary reason the dominant follicle does not proceed to ovulation is the presence of high levels of progesterone secreted by the CL, which occurs during the middle of the estrous cycle (days 5 to 17).

Follicular waves generally last 9-12 days, with some overlap. There are usually two or three waves during each estrous cycle. The dominant follicle of the last wave of the cycle becomes the follicle that ovulates. As this follicle receives the signal to ovulate, it releases its egg (ovum), and the cells that remain are reorganized into what will become the CL.

What are the products on the market for use in GnRH programs?

There are three GnRH products on the market: Cystorelin® (Merial Animal Health), Factrel® (Fort Dodge Animal Health) and Fertagyl® (Intervet). All are prescription products that must be purchased from a veterinarian.

There are also three prostaglandin products available: Lutalyse® (Pharmacia

Table 1: Comparison of 2-shot PGF and Select Synch systems

Item	2-shot PGF	Select Synch
Number of cows	537	622
Synchronization rate	58%	65%
Conception rate	61%	66%
Synchronized pregnancy rate	35%	43%

Table 2: Comparison of MGA/PGF and Select Synch systems

Item	MGA/PGF	Select Synch
No. of cows	66	75
Synchronization rate	64%	64%
Conception rate	62%	71%
Synchronized pregnancy rate	39%	45%

Table 3: Comparison of pregnancy rates in cyclic and anestrous cows when synchronized with Syncro-Mate-B or Ovsynch systems

Item	Syncro-Mate-B	Ovsynch
No. of cows:		
Cyclic	93	103
Anestrous	116	111
Synchronized pregnancy rate by postpartum status:		
Cyclic	38%	59%*
Anestrous	43%	49%

*(P<0.05).

& Upjohn), EstruMate® (Bayer) and ProstaMate™ (Phoenix Scientific). These products also must be purchased from a veterinarian or a veterinary-supply company.

What does GnRH cost?

Actual prices will vary depending on location and supplier. Prices to the producer in northern Colorado in spring 1999 were in the range of \$3.10 to \$3.50/dose. The prostaglandin products were about \$2.10-\$2.40/dose.

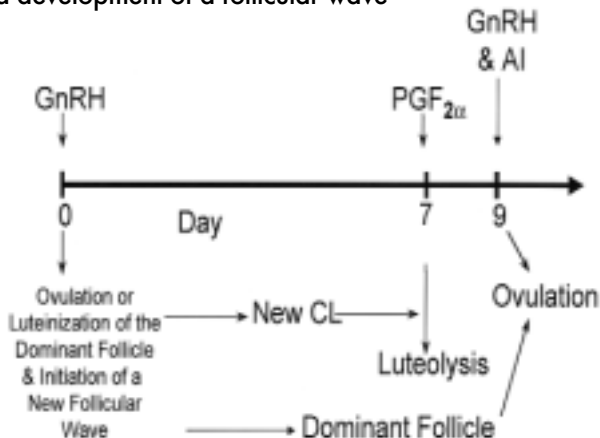
Does a GnRH injection actually cause ovulation?

Actually, the GnRH injection causes a surge of LH, as noted previously. The surge of LH induced by the GnRH injection occurs approximately seven hours after the injection, with a peak LH elevation occurring at 100 minutes (Kaltenbach et al., 1974). Work done in Florida and Canada (Macmillan and Thatcher, 1991; Twagirumungu et al., 1994a, b) demonstrated that the dominant follicle present on the ovary will ovulate in response to the induced LH surge. A study in Wisconsin, using 20 lactating dairy cows, showed that 90% of the cows ovulated a follicle after the first GnRH injection, and a new follicular wave emerged within 2-4 days after the injection (Pursley et al., 1995). Approximately 70% of suckled beef cows ovulated following the first GnRH injection (Geary et al., 2000).

When will ovulation occur with GnRH synchronization?

The synchrony of ovulation after the second GnRH injection is fairly tight, as shown by Silcox et al. (1995), who observed ovulation in lactating beef cows within 32 hours after the second GnRH

Fig. 3: Illustration of how GnRH works in the CO-Synch system to control ovulation and development of a follicular wave



injection. Pursley et al. (1995) and Silcox et al. (1995) both observed a majority of the cows (20 of 20 and 12 of 14, respectively) ovulating between 24 and 32 hours after GnRH treatment. From this work, it appears that ovulation is fairly well synchronized and rather predictable following the second GnRH injection.

How is the GnRH system different from previous synchronization systems?

We now understand much more about the estrous cycle of a cow than we did when synchronization efforts were first begun. Therefore, we are able to improve techniques for manipulating estrus and ovulation. Previous approaches for synchronization focused on managing the CL either by regressing it with injections of prostaglandins like Lutalyse, by mimicking the action of the CL by feeding progesterone-like compounds like MGA or by administering progesterone-like implants as in the Syncro-Mate-B® system.

With current and improved knowledge of follicle growth, we are now able to control both the CL and the follicle to improve synchronization efforts. Fig. 3 illustrates the mode of action of GnRH in the CO-Synch system.

The first injection of GnRH is designed to turn over the follicle wave and cause a new CL to form so that in seven days there is a functional CL and a dominant follicle when the PGF injection is given. When the suppressive effect of progesterone from the CL is removed, the dominant follicle matures; when the second GnRH injection is given on Day 9, a fertile ovulation occurs. If a second injection of GnRH were not given (as with the Select Synch system), a fertile ovulation would occur after the onset of estrus.

How do results from GnRH systems compare with results from traditional synchronization systems in beef cows?

Syncro-Mate-B vs. Ovsynch. In order to evaluate the GnRH/PGF systems in beef cows, we first evaluated pregnancy rates of cows that received the Ovsynch (220 head) or Syncro-Mate-B (216 head) system and were time-inseminated (Geary et al., 1998). Timed insemination occurred 24 hours after the second GnRH injection or 48 hours after removal of the Syncro-Mate-B implant. These cows also were exposed to 48-hour calf removal between the time that the PGF injection and second GnRH injection were given, or from the time of Syncro-Mate-B implant removal until breed-

ing. Synchronized pregnancy rates to the timed inseminations were higher for cows that received the Ovsynch system (54%) than for cows that received the Syncro-Mate-B system (42%).

Two-shot PGF vs. Select Synch. Data for this comparison was compiled by Select Sires Inc. from several universities, including data from CSU (*Select Sires Fact Sheet on Estrous Synchronization in Cattle Using GnRH and PGF*, Select Sires Inc., Plain City, Ohio). Table 1 shows this summary.

MGA/PGF vs. Select Synch. In 1996 we used 141 crossbred cows to compare the traditional MGA/PGF system in which MGA is fed for 14 days, followed 17 days later by an injection of PGF and heat detection with the Select Synch system. The cows averaged 73 days postpartum and a 5.5 body condition score (1=thin, 9=obese) at the time of PGF injections. Table 2 lists the results of the comparison.

We understand that the GnRH system has been effective in inducing anestrus cows to become pregnant to a timed injection. Explain.

Cows that pose an obstacle in synchronization are those that have not yet cycled after calving. These cows are termed *anestrus* because they are not showing estrus yet. GnRH systems currently available appear to do a reasonable job of inducing a fertile ovulation in anestrus cows.

In our work with comparing Syncro-Mate-B and Ovsynch, we collected blood samples to assay for progesterone, the hormone that indicates if the cows have returned to estrus. In this study we found that pregnancy rates were 21 percentage units higher between the two treatments among cows that were cycling (Table 3). Note that the GnRH system was just as effective as Syncro-Mate-B in jump-starting cows that were in anestrus.

When using the GnRH systems, how does fixed-time AI compare to breeding only cows observed to be in estrus?

Combined data that compared pregnancy rates of cows inseminated at a fixed time using the CO-Synch system (either 48 or 54 hours following the PGF injection) or following an observed estrus using the Select Synch system revealed similar pregnancy rates for both methods (43% and 42%, respectively). These studies were conducted at Kansas State University (54-hour timed AI; 823 head) and CSU (48-hour timed AI; 169 head) during the 1996 and 1997 breeding seasons (Grieger et al.,

1998; Thompson et al., 1998).

The conception rate of cows that received the Select Synch system was high at each location (70%), but the percentage of cows detected in estrus was low (59%). The synchronization response among cyclic (80%) and anestrus (47%) cows suggests that a higher pregnancy rate might be obtained in herds with a low percentage of anestrus cows (Thompson et al., 1998).

Based on these data, it looks like whole-herd synchronized pregnancy rates will be similar whether only cows observed in estrus are mated (Select Synch) or if all cows are mated at a fixed time (CO-Synch). The manager must then evaluate how the cost and effort of heat detection along with the use of less semen and GnRH compares to the advantages of managing breeding at a fixed time according to a preset schedule.

Are there opportunities to improve overall pregnancy rates by combining some of the present systems?

To answer this question, we'd like to refer to data already reported (Geary and Whittier, 1999) combining the Select Synch (involves heat detection) and CO-Synch (involves fixed-time AI) systems. The purpose for combining the two was to take advantage of higher conception rates generally seen among cows bred following estrus and acceptable pregnancy rates following a timed insemination.

During the 1997 breeding season, 696 cows in three locations received the Select Synch system and were artificially inseminated approximately 12 hours following observation of estrus for 72 hours following the PGF injection. At 72 hours post-PGF injection, all cows that had not yet been bred were divided into two groups to be time-inseminated at either 72 hours with a second injection of GnRH or at 84 hours with a second injection of GnRH. None of the cows used in the studies were observed for estrus prior to the PGF injection.

Forty-eight percent of the cows exhibited estrus within 72 hours following the PGF injection. The conception rate for cows bred following an observed estrus was 56%. The conception rates for the groups that received a second GnRH injection and were time-inseminated at 72 or 84 hours following the PGF injection were 21% and 24%, respectively. Thus, the overall conception rates to AI were 44% or 45% for breeding by an observed heat with timed AI at 72 or 84 hours, respectively.

During the 1998 breeding season, 682

cows at one location received the Select Synch system and were artificially inseminated approximately 12 hours following observation of estrus for 48 hours following the PGF injection. At 48 hours post-PGF, the cows that had not yet been bred were divided into two groups: one to receive a second injection of GnRH, the other to be time-inseminated at 48 or 64 hours.

None of the cows were observed for estrus prior to the PGF injection. Only 18% of the cows were observed in estrus by 48 hours post-PGF injection, but their conception rate was high (68%). Heat detection of 682 cows simultaneously is difficult, and that may have affected the low estrous response by 48 hours. The pregnancy rates of cows that were time-inseminated at 48 or 64 hours were 43% and 39%, respectively.

Taken together, these data suggest that the optimal time for high pregnancy rates to a timed AI is 48 hours following the PGF injection when a second GnRH injection is given at the time of AI. However, based on pregnancy rates of time-inseminated cows compared to those bred following an observed estrus, it would be desirable for beef producers to breed cows that show estrus before the second GnRH injection based on observed estrus, rather than waiting to 48 hours.

What about short-term calf removal and GnRH?

We addressed this question in conjunction with our evaluation of CO-Synch in 1997 and 1998. With hopes of simplifying the Ovsynch system, we evaluated the CO-Synch system to determine the effects of a timed insemination at the time of the second GnRH injection rather than handling cows a fourth time for insemination. In the same study, we also evaluated the effect of short-term calf removal on pregnancy rates using those systems.

Several studies have been conducted at CSU in which both CO-Synch and Ovsynch were used simultaneously in the same herd. By pooling the results, we found that pregnancy rates (54%) of cows receiving the CO-Synch system (606 head) were not significantly different from the pregnancy rates (58%) of cows receiving the Ovsynch system (639 head).

A subset of the cows (469 head) that received the Ovsynch or CO-Synch system were divided to receive 48-hour calf removal or no calf removal to evaluate its importance in achieving high pregnancy rates to a timed AI. When averaged across the calf-removal treatments, pregnancy rates of cows that received tempo-

Table 4: Fixed-time AI pregnancy rates of cows by synchronization and calf-removal treatments

Synchronization treatment	No. of cows	Pregnancy rate
CO-Synch + 48-hr. calf removal	119	63%
Ovsynch + 48-hr. calf removal	112	62%
CO-Synch	115	55%
Ovsynch	123	52%

Table 5: Comparison of full- and half-dose GnRH in CO-Synch and Select Synch systems in beef cows

Treatment*	Synch'd AI preg. frequency	Synch'd AI preg. rate
CO-Synch **		
50:50	38/97	38.2%
50:100	24/49	49.0%
100:50	22/52	42.3%
100:100	48/104	46.2%
Select Synch ***		
50	57/122	48.4%
100	80/130	61.5%

*For CO-Synch treatment, first GnRH injection at either 50 mg or 100 mg, followed 9 days later by either 50 mg or 100 mg of GnRH at second injection.

**P=0.65 for treatment effect.

***P=0.16 for treatment effect.

rary calf removal (62%), regardless of whether it was with the Ovsynch or CO-Synch system, were significantly higher than pregnancy rates of cows that did not receive calf removal (54%).

Short-term calf removal appears to hasten ovulation in most cows and thus may result in a tighter synchrony of ovulation. The number of animals that received the CO-Synch or Ovsynch system with or without calf removal and their timed AI pregnancy rates are listed in Table 4. The cows used in the study were also bled twice prior to synchronization to determine which cows were cycling at the time of synchronization.

Comparing the prior estrual status of cows with their pregnancy rates revealed that pregnancy rates of cyclic and anestrous cows were 70% and 58%, respectively, if they received 48-hour calf removal and 62% and 48%, respectively, if they did not receive calf removal. Thus, 48-hour calf removal is more important if producers believe a high percentage of their cows are not exhibiting normal estrous cycles at the time of synchronization.

Does day of the estrous cycle when the system begins or whether the cow has returned to estrus after calving affect the response to GnRH systems?

This question is addressed in a study published by Downing et al. (1998). In the study we used visual and electronic estrous detection to identify in which day of the estrous cycle the cows were at the start of

the Select Synch system with 57 crossbred cows. Ovaries were scanned daily with ultrasound from the day before the GnRH injection until after a response was seen from the PGF injection a week later. With the exception of cows in Day 15-17 of the estrous cycle, mean time to estrus (59.4 ± 28 hours) increased when cows were treated later in their cycle. Cows that were at Day 15-17 of their estrous cycle at the time of GnRH administration exhibited estrus 11 ± 19 hours before the PGF injection.

Simply put, cows that are in the later stages of their estrous cycle, specifically Day 15-17, are likely to show heat prior to the PGF injection and therefore need to be watched for an early heat. In practice, simply noting which cows are in heat the day of the PGF injection and inseminating them at that time will increase pregnancy in a group of cows. An alternative approach is to devise some method for "presynchronizing" the cows so that few, if any, are in the later stages of their cycle when a GnRH system is started. Several researchers are currently addressing this approach.

Can a GnRH system be used on heifers?

In 1998 we compared the Select Synch system with the MGA/PGF system in 124 yearling heifers (Doherty et al., 1998). In both treatments, heifers were detected for estrus from 12 hours prior to the PGF injection until 72 hours after. Nonresponding heifers were all inseminated beginning at 72 hours post-

injection. In this study, overall synchronized pregnancy rates were 11 percentage units higher for the MGA/PGF-treated heifers (47% for Select Synch and 58% for MGA/PGF).

To answer the question posed: Yes, GnRH can be used in heifers, but it does not appear to give results as good as those of the MGA/PGF system. However, it may provide an optional system in situations where MGA can't be fed properly or when the time frame for breeding dictates a shorter-length synchronization system.

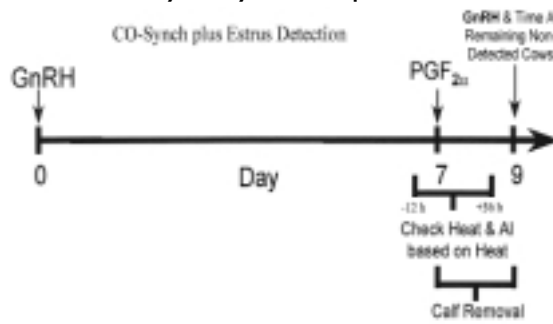
What about reduced-dose GnRH in synchronization programs?

The standard GnRH dose used in most GnRH synchronization studies to date has been based on the label dosage on Cystorelin. Cystorelin and other GnRH products are labeled “for the treatment of ovarian cysts in dairy cattle. Ovarian cysts are nonovulated follicles with incomplete luteinization, which results in nymphomania or irregular estrus” (*Compendium of Veterinary Products*, Third Edition, 1995-1996). The dosage for cystic-follicle therapy is 100 milligrams (mg) of GnRH. As investigations were done with GnRH for synchronization systems, it was logical to use that dose.

The injection concentration of Cystorelin is such that 2 milliliters (mL) are given to deliver 100 mg of the compound. Questions have arisen concerning injection errors with such a small volume. Under practical farm and ranch conditions, cows are frequently injected while crowded into an alley, rather than individually restrained in a squeeze chute or head catch. This can lead to a less-than-precise amount of compound actually being given to or absorbed by the animal. It also appears that in some instances a small amount of the injection may ooze from the injection site and not be absorbed by the animal.

Work done with dairy cows (Fricke et al, 1998) showed no difference in synchrony or pregnancy response when 50 mg of GnRH were used. These data have led to questions about a reduced dose of GnRH in beef synchronization systems. During the breeding seasons of 1999 and 2000, we addressed this question by evaluating either 100-mg or 50-mg doses in both CO-Synch and Select Synch systems. The CO-Synch system involves two injections of GnRH; therefore, we had the possibility of four sequences for evaluating half-dose GnRH in this system. Table 5 shows the results from our 1999 studies. There were no significant differences in synchronized pregnancy rates when a half dose of GnRH was used.

Fig. 4: Illustration of CO-Synch system coupled with estrous detection



(Approximately 5%-8% of the cows will show estrus before or near the time of the PGF injection. It is important to identify these animals and inseminate them at that time (TAI=Timed AI). Continuing estrus detection until 36 hours following PGF injection will increase pregnancy rates. All remaining animals would then be inseminated at the fixed time of 48 hours post PGF injection.)

However, there was a tendency for fewer cows injected with 50 mg of GnRH to be pregnant. The data from the 2000 studies are not yet available.

Additional data on this question was presented recently by the University of Kentucky (Funk and Anderson, 2000). In their study, cows were synchronized with CO-Synch and treated with either 100 mg or 50 mg of GnRH at both injections. Conception rates to fixed-time inseminations were significantly lower for cows treated with 50 mg (32%; 76 of 239) compared to 100 mg (42%; 106 of 255).

At this point we are cautious about making a recommendation on reducing the dose of GnRH in synchronization programs for beef cows, even though research in dairy cows showed no difference in response. For now, we suggest that beef producers continue to use 100 mg in GnRH synchronization systems.

What recommendations would you give cattlemen to help them prepare to use GnRH in a synchronization system? Are there any precautions?

Unfortunately, one of the drawbacks of most synchronization systems available today is the need to handle the cows repeatedly in a short period of time. This can lead to cows becoming “sour” and hard to work. Principles of good cattle handling and well-designed working facilities become important in these situations. If calf removal can be used, hold the calves in a place that will assist in getting the cows gathered and through the chutes for the second GnRH injection and AI if using CO-Synch.

Coupling estrous detection with fixed-time AI may be warranted when feasible. Some cows will show heat at or near the time of PGF injection, so don't overlook and miss those cows. If heat detection and AI are done from the day of PGF injection

up to 36 hours following, then non-detected cows inseminated at 48 hours, overall results generally will be higher than a strict fixed-time AI at 48 hours.

Shop around for GnRH products. There are now three different manufacturers in the market, which has helped bring down the cost of GnRH. Remember that GnRH is a prescription product and must be obtained from a veterinarian. Consider veterinary-supply businesses when trying to locate a cost-competitive source.

There are indications that some sires have higher conception rates when used in timed insemination programs than do other sires. The reasons for this are still unclear, but they likely relate to the length of time the semen remains viable in the female reproductive tract. Producers using timed AI programs should be aware of this and watch as new information unfolds relative to sire differences. Many AI companies will provide useful advice concerning bulls that have worked well in timed-insemination programs.

Finally, as with all other synchronization programs, GnRH systems will not overcome inadequate nutrition, improper semen handling or poor insemination technique. It is important that management of all aspects of the cow herd is in order before one can expect success with a synchronized AI program.

What do you recommend for synchronizing beef cows?

Based on studies we have conducted with CO-Synch, we believe it is a reasonable approach for synchronizing beef cows. Due to manufacturing problems with Syncro-Mate-B, it is currently not available. We believe that CO-Synch will work as a replacement for Syncro-Mate-B in fixed-time AI programs for beef cows. We recommend calf removal with the CO-Synch system in situations where it can be employed. Not only does calf re-

removal increase pregnancy, but it actually makes the system easier to apply; when calves are sorted off to give the PGF injection, they are left off the cows so they do not have to be resorted for the second injection of GnRH and fixed-time AI. A diagram illustrating this system is shown in Fig. 4.

We have seen 40%-45% pregnancy rates with no heat detection and fixed-

time breeding at 48 hours, along with the second GnRH injection. Heat detection and breeding from -12 hours to +36 hours likely will increase pregnancy by 3%-5%. Using calf removal will typically increase pregnancy rates by 5%-8%.



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