The Latest in Estrous Synchronization in Beef Cattle

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To effectively utilize artificial insemination in the beef cattle industry and achieve the genetic progress desired by many beef cattle producers, regulation of the estrous cycle is extremely important. In the past, extensive research has evaluated prostaglandins and progestin compounds such the SyncroMate B® implant system. More recently, extensive use of the MGA/prostaglandin synchronization system has been utilized with beef heifers.

Recent research has evaluated the potential of utilizing GnRH in combination with prostaglandin to synchronize estrus in cows and heifers. The following is a brief review of the research data collected to date.

What are the objectives of using GnRH in a synchronization program?

The newest synchronization strategies utilize gonadotropin-releasing hormone (GnRH) in tandem with prostaglandin $F_{2\alpha}$ (PGF) in order to manage follicular growth as well as the CL. The objectives of these strategies are to: 1) tighten the synchrony of estrus to increase the efficiency of estrous detection; and 2) control the time of ovulation for fixed-time AI, thereby eliminating the need for estrus detection.

What is GnRH?

GnRH is a decapeptide produced by neurons in the hypothalamus that stimulates the release of luteinizing hormone (LH) and follicle stimulating hormone (FSH) from the anterior pituitary. Of primary importance to estrous synchronization and fertility in cattle is the GnRH-induced preovulatory surge of LH that occurs at the onset of estrus and causes ovulation 27 h later. It has been known for some time that the preovulatory surge of LH can be mimicked with administration of GnRH agonists. Several GnRH analogues (gonadorelin - *Cystorelin, Factrel, Fertagyl*) and agonists (buserelin - *Receptal;* fertirelin acetate - *Ovulyse*) are commercially available and their potencies when administered to cattle have been compared. An intramuscular injection of GnRH will cause a detectable increase in LH and FSH in the blood within 15 minutes that peaks in 2-3 hr mimicking the preovulatory surge.

How does GnRH work?

The effects on the ovary of exogenous GnRH at different times of the cycle have been reviewed recently. It is evident that GnRH inhibits estrus for at least 6 d after its administration. Twagiramungu *et al.*[9] proposed a model to explain the mechanism by which GnRH acts on follicular dynamics to cause this suppression of estrus. In cows without a functional CL, or those in early luteal phase (d 4-7), GnRH-induced LH release caused ovulation of the dominant follicle. However, when GnRH is given during the mid or late luteal phase at a time when concentrations of progesterone are elevated, the largest follicles undergo partial luteinization or atresia. Using daily blood sampling and ultrasonography, Twagiramungu *et al.*[10] reported that GnRH-induced ovulation was dependent on progesterone concentrations at the time of injection. When serum progesterone concentrations were high, no ovulation occurred, but when progesterone was low the dominant follicle ovulated. However, unpublished data by Pursley[3] reported that lactating dairy cows ovulated in response to GnRH during the early (100%), mid (70%) and late (100%) luteal phase of their cycles. This suggested that cows did ovulate at times of high progesterone concentrations (d 10-15). These studies indicate that exogenous GnRH eliminates the dominant follicle through ovulation, luteinization, or atresia, thereby suppressing estrus for approximately 6 d after administration.

These data also support the concept that elimination of the dominant follicle with GnRH initiates a newly synchronized follicular wave in cycling cows and heifers. The mature and/or newly induced CL can then be regressed with PGF 6 d after GnRH. This 6-d interval would allow a newly induced CL time to mature to a point at which it would regress in response to PGF. Thatcher *et al.*[8] reported a 7-d interval between GnRH and PGF resulted in greater synchrony of estrus in heifers (96% in 4 d) than was demonstrated for an 8-d interval (60%). GnRH caused ovulation, induced a new or accessory CL, and initiated a new follicular wave in 18 of 20 dairy cows and in 13 of 24 dairy heifers. PGF given to cows 7 d after GnRH in this study ovulated within an 8-h window (24-32 h after GnRH). It can be concluded from these reports and others that GnRH

given 7 d prior to PGF tightened synchrony of estrus and decreased the time needed for estrous detection. However, what is its effect on fertility? Experiments addressing this question were designed to synchronize estrus in cows followed by AI according to observed estrus or at a fixed time after treatment.

GnRH - PGF: AI after detected estrus

Conception rate of heifers bred according to observed estrus after GnRH and PGF given 7 d (57%) or 8 d (62%) apart was not different. In a similar design, postpartum beef cows were treated with either saline or GnRH at d 0 followed by PGF at d 6. Cows were inseminated after detected estrus for a 10 d period beginning at the time of GnRH or saline treatment. A greater number of GnRH-treated cows were observed in estrus (83%) between d 6 and 10 compared to saline treated cows (50%). Over the 10-d period, the estrus response (88% vs 85%) and conception rate (71% vs 70%) were similar between GnRH and saline treatments. Coleman *et al.*[1] compared a double injection of PGF (11 d apart) to a treatment of GnRH (d 0) and PGF (d 7) in beef cows. All cows were inseminated after observed estrus. Estrous response (68%) and conception rate (65%) were not different between the two groups. It is not clear from this study if estrus was observed during the entire treatment period or only after the second PGF injection. Collectively, these studies indicate that using GnRH followed in 6-8 d by PGF will increase estrous synchrony and not affect fertility in cycling cows and heifers compared to using PGF alone.

GnRH - PGF - GnRH: Timed AI

A similar protocol with the addition of a second injection of GnRH after PGF has produced some favorable results using fixed-time AI in cattle. The administration of GnRH at the appropriate time after regressing the CL will induce a preovulatory surge of LH prior to the endogenous surge. If timing of the LH surge can be controlled, and therefore the time of ovulation, success of timed AI could be achieved.

To examine the optimal interval between PGF and the second GnRH injection, Pursley *et al.*[4] gave lactating dairy cows PGF either 7, 8 or 9 d after an initial GnRH injection. A second injection of GnRH was administered to all cows on d 9, thereby creating intervals of 48, 24 or 0 h between PGF and the second GnRH. All cows were time inseminated 24 h after the second GnRH on d 10. Conception rate was higher for cows given PGF 48 (55%) and 24 h (46%) before GnRH, than cows given GnRH and PGF at the same time (11%). These authors also reported a 50% conception rate for lactating dairy cows in a second experiment using a protocol of: GnRH (d 0) - PGF (d 7) - GnRH (d 9) - AI (d 10). These were promising results as normal conception rates for this herd in previous years were between 40 and 50%.

Stevenson *et al.*[7] treated dairy cows and heifers with GnRH (d 0) - PGF (d 7) and administered a second GnRH at 30 h after PGF. All animals were time-inseminated 18-19 h after GnRH. Conception in these cows (35%) tended to be lower than a control group receiving a single PGF injection and bred according to detected estrus (47%). Roy and Twagiramungu[5] compared beef cows treated with GnRH (d 0) - PG (d 6) - GnRH (d 8) and time-AI (15 h post-GnRH) to cows receiving GnRH (d 0) - PGF (d 6) and inseminated according to detected estrus. The conception rate for females time-bred (62%) was not different than those bred according to estrus (70%). Pursley *et al.* (33) compared conception rates of dairy cows inseminated 0, 8, 16, 24, and 32 h after the second GnRH injection. There was a trend for greater conception at 16 h (44%), and for lower conception at 32 h (32%). Schmitt *et al.*[6] reported a lower pregnancy rate (26%) in dairy heifers receiving GnRH (d 0) - PG (d 7) - GnRH (d 8) - timed AI (15 h) compared to heifers treated with GnRH (d 0) - PGF (d 7) and inseminated according to estrus (49%). In a second experiment, heifers receiving GnRH (d 0) - PGF (d 7) - GnRH (d 9) responded with a comparable pregnancy rate (45%) to controls receiving GnRH (d 0) - PGF (d 7) and inseminated according to estrus (48%). Therefore, delaying the second injection of GnRH for 48 h after PGF improved pregnancy rate in dairy heifers. However, conception rate was lower in the timed AI group compared to controls (46% vs 61%).

There are also some recent data suggesting a positive effect on anestrous cows. Forbes and co-workers[2] reported an increase in conception rate (69% vs 27%) and pregnancy rate (21% vs 4%) of anestrous cows treated with GnRH (d 0) - PGF (d 7) compared to controls (double injection of PGF).

Are there any field evaluation data of the GnRH-PG system?

Four herds of predominantly crossbred cows (n= 911) at three locations were allotted randomly to two treatments and one control (Figure 1): 1) 100 μg of GnRH
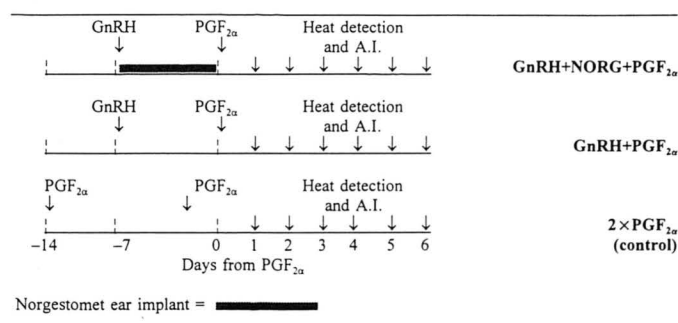

Figure 1. Experimental protocol for two new estrus-synchronization treatments.

and a 6-mg norgestomet ear implant on day -7 and 25 mg of $PGF_{2\alpha}$ and implant removal on day 0 ($GnRH+NORG+PGF_{2\alpha}$); 2) 100 µg of GnRH on day -7 and 25 mg of $PGF_{2\alpha}$ day 0 ($GnRH+PGF_{2\alpha}$); and 3) 25-mg injections of $PGF_{2\alpha}$ on days -14 and 0; $2xPGF_{2\alpha}$) (control). Three blood samples were collected (days -14, -7, and 0) before the last $PGF_{2\alpha}$ injection to determine estrus-cycling status. If any one of the three samples contained ≥1 ng/ml serum progesterone, then the cows were assumed to be cycling. Cows were observed for estrus twice daily (4 hours each) during 144 hours after $PGF_{2\alpha}$. All cows were inseminated 12 to 14 hours after first detected standing estrus. Body condition score was assessed at the time of $PGF_{2\alpha}$ injection, and pregnancy was diagnosed by transrectal ultrasonography between 32 and 51 days after AI. Conception rate was defined as the proportion of cows detected in estrus and inseminated during 144 hours after $PGF_{2\alpha}$ that became pregnant. Pregnancy rate was defined as the proportion of treated cows became pregnant.

The results of this experiment were as follows: Body condition scores ranged from 1 (thinnest) to 6.5, with an average of 4.5 on a 1 to 9 scale. In addition, days postpartum at the onset of the breeding season ranged from 21 to 108, with an overall average of 72 days across all herds. The combination of somewhat lower body condition scores, fewer days postpartum, and the lack of spring pasture that may have reduced estrus, conception, and pregnancy rates in herds 3 and 4.

The percentages of cows that exhibited standing estrus were greater (P<.05) in the two treatments than in controls. The $GnRH+NORG+PGF_{2\alpha}$ treatment had 51% and the $GnRH+PGF_{2\alpha}$ treatment had 27% more cows showing heat than the control. Although the treatments had no statistical significant effect on conception rate (Table 1), pregnancy rates were greater (P<.05) in the two treatments than in the control.

Based on the three blood samples, 54.8% of the females were cycling at the time of the $PGF_{2\alpha}$ injection. Within the cycling cows, conception and pregnancy rates were not different between treatments and control (Table 2). The $GnRH+NORG+PGF_{2\alpha}$ treatment induced both the earliest and tightest synchrony of estrus. In that

Table 1. Expression of Estrus, Conception, and Pregnancy Rates[a]

Herd	No.	Estrus, %			Conception, %			Pregnancy, %			BCS[b]	Days[b]
		A	B	C	A	B	C	A	B	C		
1	206	92.1	76.5	60.9	67.2	75.5	52.4	61.9	57.8	31.9	4.7	81
2	266	89.5	79.8	71.6	57.1	69.0	68.2	51.2	55.1	48.9	4.6	73
3	329	54.6	44.4	25.7	53.4	55.3	57.1	28.7	24.1	14.7	4.5	68
4	110	38.9	23.5	25.0	57.1	37.5	55.6	22.2	8.8	13.9	3.8	64
All	911	71.0[x]	59.7[y]	47.0[z]	58.9	65.7	60.6	41.6[x]	39.0[x]	28.5[y]	4.5	72

[a]A = $GnRH+NORG+PGF_{2\alpha}$; B = $GnRH+PGF_{2\alpha}$; and C = $2xPGF_{2\alpha}$.
[b]BCS = body condition score and days postpartum at beginning of the breeding season (time of $PGF_{2\alpha}$ injection)
[x,y,z](P<.05)

Table 2. Reproductive Traits of All Cows Based on Concentrations of Progesterone

Cycling status[a]	Treatment		
	$GnRH+NORG+PGF_{2\alpha}$	$GnRH+PGF_{2\alpha}$	$2\times PGF_{2\alpha}$
Anestrus, %	51.2	38.3	45.6
No. of cows	153	116	140
Estrus, %	51.0	30.2	15.7
Conception rate, %	58.4	68.6	27.3
Pregnancy rate, %	54.1	20.7	4.2
Cycling, %	48.8[b]	61.7[c]	54.4[b,c]
No. of cows	146	187	166
Estrus, %	91.8	79.1	74.1
Conception rate, %	59.0	64.6	66.7
Pregnancy rate, %	54.1	50.8	49.4

[a]If any one of three blood serum samples contained high (≥ 1 ng/ml) progesterone then the cows were assumed to be estrus-cycling before $PGF_{2\alpha}$ injection. Otherwise, the cows were assumed to be anestrus because progesterone was low (< 1 ng/ml)in all three blood serum samples.
[b,c](P < .01).

treatment, 50.5% of the cows showed detectable estrus between 24 and 48 hours after the $PGF_{2\alpha}$ injection, compared to 32.4% in the $GnRH+PGF_{2\alpha}$ and 16.1% in the $2xPGF_{2\alpha}$ (control) group.

Summary

In summary, GnRH will initiate a new follicular wave in 80 to 100% of cycling cows, although it is somewhat less effective in heifers. Injection of PGF 7 d after GnRH regresses the CL to allow emergence of a healthy preovulatory follicle. GnRH (d 0) - PGF(d 7) increases the precision of estrous synchrony in cows and heifers. It may also induce some anestrous cows to initiate cycling. Perhaps more significantly, a protocol of GnRH (d O) - PGF (d 7) - GnRH (d 9) in combination with fixed-time AI (16 to 24 h post-GnRH) has potential to eliminate estrous detection without compromising conception rate.

References

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CVM UPDATE

FDA, Center for Veterinary Medicine
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June 2, 1997

"FDA ORDER PROHIBITS EXTRA-LABEL USE OF FLUOROQUINOLONES AND GLYCOPEPTIDES"

In the May 22, 1997 <u>Federal Register</u>, the Food and Drug Administration (FDA) issued an order prohibiting the extra-label use of fluoroquinolones and glycopeptides. The Agency issued this order because it believes that some extra-label uses of fluoroquinolones and glycopeptides in food-producing animals are capable of increasing the level of drug resistant zoonotic pathogens (pathogens that are infective to humans) in treated animals at the time of slaughter. In the order, FDA determined that some extra-label uses of fluoroquinolone and glycopeptide drugs in food-producing animals likely will cause an adverse event, which constitutes a finding under the Animal Medicinal Drug Use Clarification Act of 1994 (the AMDUCA) that extra-label use of these drugs in food animals presents a risk to the public health. Therefore, the Agency issued the order of prohibition.

On October 22, 1994, the President signed the AMDUCA into law. The AMDUCA amended the Federal Food, Drug, and Cosmetic Act to allow licensed veterinarians to prescribe extra-label uses of approved animal drugs and human drugs in animals. Section 2(a)(4)(D) of the AMDUCA provides that the Agency may prohibit an extra-label drug use in animals if, after affording an opportunity for public comment, the Agency finds that such use presents a risk to the public health.

FDA intends to prohibit by order the extra-label use of fluoroquinolones and glycopeptides in food-producing animals because the Agency has determined that use of these drugs other than for the approved label indication in food-producing animals meets the criteria for prohibition in the AMDUCA. Under this order, fluoroquinolones and glycopeptides were added to the list of those drugs already prohibited for extra-label use. The revised list states that the following drugs, families of drugs, and substances are prohibited for extra-label animal and human drug uses in food-producing animals.

> (1) Chloramphenicol; (2) Clenbuterol; (3) Diethylstilbestrol (DES); (4) Dimetridazole; (5) Ipronidazole; (6) Other nitroimidazoles; (7) Furazolidone (except for approved topical use); (8) Nitrofurazone (except for approved topical use); (9) Sulfonamide drugs in lactating dairy cattle (except approved use of sulfadimethoxine, sulfabromemthazine, and sulfaethoxypyridazine); (10) Fluoroquinolones; and (11) Glycopeptides.

The order of prohibition is effective August 20, 1997. Written comments on this order are to be submitted by July 21, 1997, to the Dockets Management Branch (HFA-305), Food and Drug Administration, 12420 Parklawn Dr., Room 1-23, Rockville, MD 20857. Comments should contain the Docket Number, 97N-0172.

Information on this prohibition is contained in the <u>Federal Register</u>, which is available for review or downloading on CVM's Internet Website at **http://www.cvm.fda.gov/.** Paper copies are available from the Communications Staff, FDA/Center for Veterinary Medicine, HFV-12, 7500 Standish Place, Rockville, MD 20855, telephone 301-594-1755.

Questions about this order may be directed to Richard L. Arkin, Center for Veterinary Medicine (HFV-238), Food and Drug Administration, 7500 Standish Place, Rockville, MD 20855, 301-594-1737.

Issued by FDA, Center for Veterinary Medicine, Office of Management and Communications.