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EMERGING PROTOCOLS TO SYNCHRONIZE ESTRUS
IN REPLACEMENT BEEF HEIFERS AND POSTPARTUM COWS

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INTRODUCTION. The beef cattle industry has seen rapid gains in economically desirable traits largely due to the selection and expanded use of genetically superior sires made available through artificial insemination (AI). Recent surveys indicate, however that less than 5 percent of the beef cows in the United States are bred by AI, and only half of the cattle producers who practice AI use any form of estrus synchronization to facilitate their AI programs. The inability to predict time of estrus for individual cows or heifers in a group often makes it impractical to use AI because of the labor required for detection of estrus. Available procedures to control the estrous cycle of the cow can improve reproductive rates and speed up genetic progress. These procedures include synchronization of estrus in cycling females, and induction of estrus accompanied by ovulation in heifers that have not yet reached puberty or among cows that have not returned to estrus after calving.

The following protocols and terms will be referred to throughout this manuscript.

Protocols:

PG: Prostaglandin $F_{2\alpha}$ (PG; Lutalyse, Estrumate, ProstaMate, InSynch).

MGA-PG: Melengestrol acetate (MGA; .5 mg/hd/day) is fed for a period of 14 days with PG administered 17 to 19 days after MGA withdrawal.

GnRH-PG (Select Synch): Gonadotropin-releasing hormone injection (Cystorelin, Factrel, Fertagyl) followed in 7 days with an injection of PG.

MGA-GnRH-PG (MGA[®] Select): MGA is fed for 14 days, GnRH is administered 10 or 12 days after MGA withdrawal, and PG is administered 7 days after GnRH.

7-11 Synch: MGA is fed for 7 days, PG is administered on the last day MGA is fed, GnRH is administered 4 days after the cessation of MGA, and a second injection of PG is administered 11 days after MGA withdrawal.

Terms:

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

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

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

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

There are several advantages to a successful estrus synchronization program. These include: 1) Cows or heifers are in estrus during a predictable interval, which allows for artificial insemination, embryo transfer or other planned reproductive techniques; 2) The time required to detect estrus is reduced, which in turn decreases labor expense associated with the breeding program; 3) Cattle will conceive earlier during the breeding period; and 4) Calves will be older and weigh more at weaning.

To avoid problems when using estrus synchronization, females should be selected for a program when the following conditions are met: 1) Adequate time has elapsed from calving and the time synchronization treatments are implemented (a minimum of 40 days postpartum at the beginning of treatment is suggested); 2) Cows are in average or above-average body condition (scores of at least 5 on a scale of 1 to 9); 3) Cows experience minimal calving problems; 4) Replacement heifers are developed to prebreeding target weights that represent at least 65 percent of their projected mature weight; and 5) Reproductive tract scores (RTS) are assigned to heifers no more than two weeks before a synchronization treatment begins (scores of 3 or higher on a scale of 1 to 5) and at least 50 percent of the heifers are assigned a RTS of 4 or 5 (Patterson et al., 2000a).

DEVELOPMENT OF METHODS TO SYNCHRONIZE ESTRUS. The development of methods to control the estrous cycle of the cow has occurred in five distinct phases. The physiological basis for estrus synchronization followed the discovery that progesterone inhibited preovulatory follicular maturation and ovulation. Regulation of estrous cycles was believed to be associated with control of the corpus luteum, whose life span and secretory activity are regulated by trophic and lytic mechanisms. **Phase I** included efforts to prolong the luteal phase of the estrous cycle or to establish an artificial luteal phase by administering exogenous progesterone. Later, progestational agents were combined with estrogens or gonadotropins in **Phase II**; whereas **Phase III** involved prostaglandin $F_{2\alpha}$ (PG) and its analogs as luteolytic agents. Treatments that combined progestational agents with PG characterized **Phase IV**.

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

A single injection of gonadotropin-releasing hormone (GnRH) to cows at random stages of their estrous cycles causes release of luteinizing hormone leading to synchronized ovulation or luteinization of most large dominant follicles. Consequently, a new follicular wave is initiated in all cows within 2 to 3 days of GnRH administration. Luteal tissue that forms after GnRH administration is capable of undergoing PG-induced luteolysis 6 or 7 days later (Twagiramungu et al., 1995). This method will be referred to as the GnRH-PG protocol throughout this manuscript. The GnRH-PG protocol increased estrus synchronization rate in beef (Twagiramungu et al., 1992a,b) and dairy (Thatcher et al., 1993) cattle. A drawback of this method is that approximately 5 to 15% of the cows

are detected in estrus on or before the day of PG injection, thus reducing the proportion of females that are detected in estrus and inseminated during the synchronized period (Kojima et al., 2000).

Synchronization of estrus and ovulation with the GnRH-PG-GnRH protocol.

Administration of PG alone is commonly utilized to synchronize an ovulatory estrus in cycling cows. However, this method is ineffective in anestrous females and variation among animals in the stage of the follicular wave at the time of PG injection directly contributes to the variation in onset of estrus during the synchronized period (Macmillan and Henderson, 1984; Sirois and Fortune, 1988). Consequently, the GnRH-PG-GnRH protocol was developed to synchronize follicular waves and timing of ovulation. The GnRH-PG-GnRH protocol for fixed-time AI results in development of a preovulatory follicle that ovulates in response to a second GnRH-induced LH surge 48 hours after PG injection. Addition of a GnRH injection 48 hours after PG has been given the trademark, Ovsynch (Pursely et al., 1995). Ovsynch was validated recently as a reliable means of synchronizing ovulation for fixed-time AI in lactating dairy cows. Time of ovulation with Ovsynch occurs between 24 to 32 hours after the second GnRH injection and is synchronized in 87 to 100% of lactating dairy cows. Pregnancy rates among cows that were inseminated at a fixed time following Ovsynch ranged from 32 to 45%, rates comparable to controls. The Ovsynch protocol, however, did not effectively synchronize estrus and ovulation in dairy heifers (35% pregnancy rate compared with 74% in PG controls).

Recently, variations of the Ovsynch protocol (CO-Synch and Select Synch) were tested in postpartum beef cows (Figure 1). It is important to understand that treatment variations of Ovsynch currently being used in postpartum beef cows have not undergone the same validation process that Ovsynch underwent in lactating dairy cows. At this point we do not know whether response in postpartum beef cows to the protocols outlined in Figure 1 is the same or different from lactating dairy cows due to potential differences in follicular wave patterns. Differences in specific response variables may include: a) the relative length of time to ovulation from the second GnRH injection; b) the anticipated range in timing of ovulation; and c) the degree of ovulation synchrony that occurs.

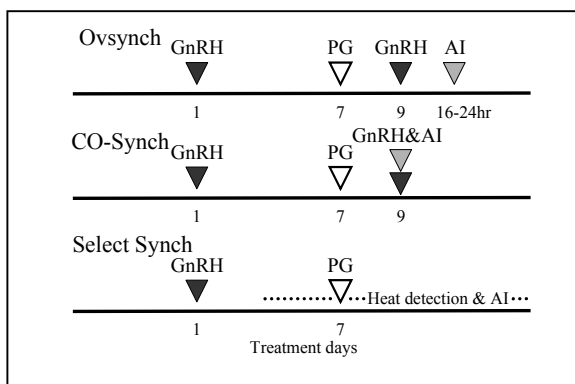


Figure 1. Methods currently being used to synchronize ovulation in postpartum beef cows: Ovsynch, CO-Synch and Select Synch.

Two variations from Ovsynch being used most extensively in postpartum beef cows are currently referred to as CO-Synch and Select Synch. CO-Synch (Geary et al., 1998) is

similar to Ovsynch in that timing and sequence of injections are the same and all cows are inseminated at a fixed time. CO-Synch differs from Ovsynch, however, in that cows are inseminated when the second GnRH injection is administered, compared to the recommended 16 hours after GnRH for Ovsynch treated cows. Select Synch (Downing et al., 1998) differs too, in that cows do not receive the second injection of GnRH and are not inseminated at a fixed time. Cows synchronized with this protocol are inseminated 12 hours after detected estrus. It is currently recommended for Select Synch treated cows that detection of estrus begin as early as day 4 after GnRH injection and continue through day 5 after PG (Kojima et al., 2000). Select Synch, similar to Ovsynch, was less effective than the melengestrol acetate (MGA)-PG protocol in synchronizing estrus in beef heifers (Stevenson et al., 1999).

THE MGA PROGRAM. This manuscript reviews recently developed methods using MGA to control estrous cycles of cows or heifers in breeding programs involving natural service or artificial insemination. Four methods will be outlined for using the melengestrol acetate (MGA®Premix, Pharmacia Animal Health, Kalamazoo, Mich.) program to facilitate estrus synchronization in heifers or cows. The choice of which system to use depends largely on a producer's goals. Melengestrol acetate is the common denominator in each of the systems presented here. MGA is an orally active progestin. When consumed by cows or heifers on a daily basis, MGA will suppress estrus and prevent ovulation. MGA may be fed with a grain or a protein carrier and either top-dressed onto other feed or batch mixed with larger quantities of feed. MGA is fed at a rate of 0.5 mg/animal/day. The duration of feeding may vary between protocols, but the level of feeding is consistent and critical to success. Animals that fail to consume the required amount of MGA on a daily basis may prematurely return to estrus during the feeding period. This can be expected to reduce the synchronization response. Therefore, adequate bunk space must be available so that all animals consume feed simultaneously.

Animals should be observed for behavioral signs of estrus each day of the feeding period. This may be done as animals approach the feeding area and before feed distribution. This practice will ensure that all females receive adequate intake. Cows and heifers will exhibit estrus beginning 48 hours after MGA withdrawal, and this will continue for 6 to 7 days. It is generally recommended that females exhibiting estrus during this period not be inseminated or exposed to natural service because of the reduced fertility females experience at the first heat after MGA withdrawal.

METHOD 1: MGA WITH NATURAL SERVICE. The simplest method involves using bulls to breed synchronized groups of females. This practice is especially useful in helping producers make a transition from natural service to artificial insemination. In this process, cows or heifers receive the normal 14-day feeding period of MGA and are then exposed to fertile bulls about 10 days after MGA withdrawal (Figure 2).

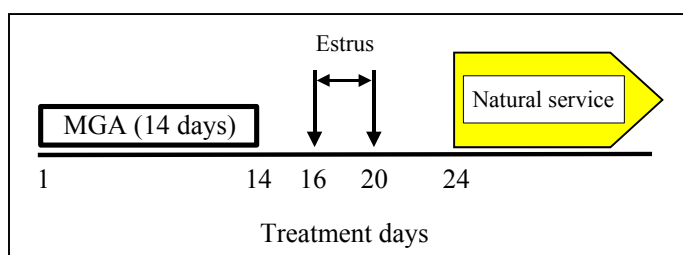


Figure 2. MGA and natural service (adapted from Patterson et al., 2000b).

This system works effectively, however, careful attention to bull to female ratios should be observed. It is recommended that 15 to 20 synchronized females be exposed per bull. Age and breeding condition of the bull and results of breeding soundness examinations should be considered carefully.

METHOD 2: MGA + PROSTAGLANDIN. A more precise means of estrous cycle control involves the combination of MGA with prostaglandin $F_{2\alpha}$. Prostaglandin $F_{2\alpha}$ (PG) is a luteolytic compound normally secreted by the uterus of the cow. Prostaglandin $F_{2\alpha}$ can induce luteal regression but cannot inhibit ovulation. When PG is administered in the presence of a functional corpus luteum (CL) during days 6 to 16 of the estrous cycle, premature regression of the CL begins and the cow returns to estrus.

In this program, prostaglandin should be administered 19 days after the last day of MGA feeding. This treatment places all animals in the late luteal stage of the estrous cycle at the time of injection, which shortens the synchronized period and maximizes conception rate (Figure 2). Although a 19-day interval is optimal, 17- to 19-day intervals produce acceptable results and provide flexibility for extenuating circumstances (Brown et al., 1988; Lamb et al., 2000). Four available PG products for synchronization of estrus in cattle can be used after the MGA treatment: Lutalyse[®], ProstaMate[®], InSynch[®], or Estrumate[®]. Label-approved dosages differ with each of these products; carefully read and follow directions for proper administration before their use.

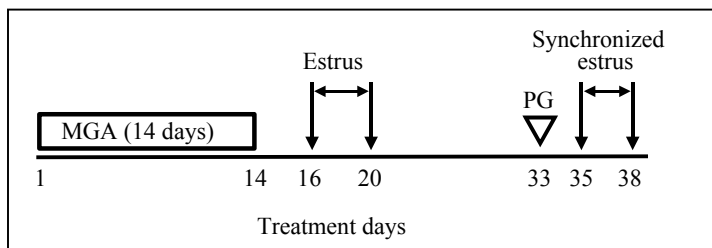


Figure 2. The MGA-PG protocol (adapted from Brown et al., 1988; Lamb et al., 2000).

Figure 3 (Patterson et al., 2000b) illustrates the distribution of estrus comparing the MGA-PG system to an MGA-only system. The combined MGA-PG system is best suited for use with AI programs because of the high degree of synchrony that can be achieved, which decreases the amount of time required for detection of estrus. Under natural mating conditions there may be an advantage to distribute estrus over several additional days to prevent overworking of bulls used in these programs.

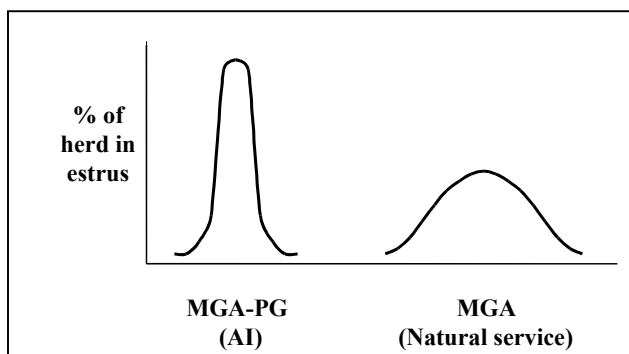


Figure 3. Distribution of estrus comparing the MGA-PG system to an MGA-only system (adapted from Patterson et al., 2000b).

Table 1 provides a summary of field trials involving heifers where MGA was used in conjunction with natural service or MGA-PG was used prior to AI (Patterson et al., 2000b). One of the major advantages in using MGA to control estrous cycles of cattle, as seen from the data presented in Table 1, is the flexibility in matching specific synchronization protocols with the particular management system involved.

Table 1. Summary of estrus synchronization field trials using MGA prior to natural service or MGA-PG prior to AI (Patterson et al., 2000b).

Breeding program	Number of heifers	Estrus response		Synchronized conception rate		Synchronized pregnancy rate	
		No.	%	No.	%	No.	%
Natural service	1749	-----		-----		1151/1749	66
AI	4245	3354/4245	79	2414/3354	72	2414/4245	57

METHOD 3: MGA[®] SELECT. The MGA[®] Select treatment (Wood et al., 2001; Figure 4) is useful in maximizing estrus response and reproductive performance in postpartum beef cows. The MGA[®] Select protocol is a simple program that involves feeding MGA for 14 days followed by an injection of GnRH (Cystorelin[®], Facrel[®], or Fertagyl[®]) on day 26 and an injection of PG on day 33. The addition of GnRH to the 14-19 day MGA-PG protocol improves synchrony of estrus, while maintaining high fertility in postpartum beef cows.

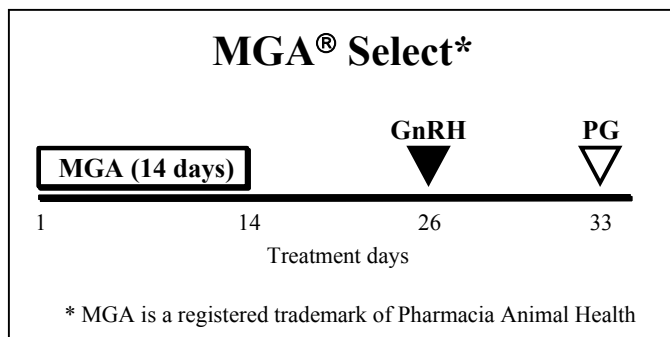


Figure 4. The MGA[®] Select protocol. MGA is fed for a period of 14 days followed in 12 days (day 26) by an injection of GnRH, and PG 19 days after MGA withdrawal (day 33).

We conducted experiments during the spring 2000 and 2001 breeding season to compare the 14-19 day MGA-PG protocol with or without the addition of GnRH on day 12 after MGA withdrawal and 7 days prior to PG in postpartum suckled beef cows (Patterson et al., 2001; Figure 5). These experiments were conducted at the University of Missouri Thompson Farm at Spickard, MO.

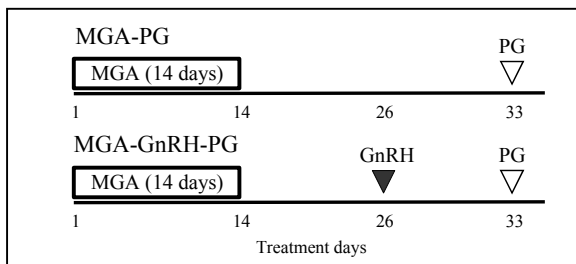


Figure 5. Cows were fed MGA for 14 days; 19 days after MGA withdrawal PG was administered to all cows. GnRH was administered to 1/2 of the cows 7 days prior to PG (Patterson et al., 2001).

The following tables provide a summary of the results from the study conducted during the 2001 breeding season. Table 2 provides a summary of the number of cows within age group by treatment, the average number of days postpartum and body condition score on the first day of MGA feeding, and the percentage of cows that were cycling prior to the treatment with MGA began. Cyclicity status was determined based on two blood samples for progesterone obtained 10 days before and on the first day of MGA.

Table 2. Number of cows within age group per treatment, days postpartum, body condition and cyclicity status at the time treatment with MGA began ¹ (Patterson et al., unpublished data).					
Treatment	Age group (yrs)	No. of cows	Days postpartum	Body condition score	Cycling (%)
MGA-PG	2, 3 & 4	52	47	5.2	35
	5+	48	39	5.2	15
	Total	100	44	5.2	40
MGA-GnRH-PG	2, 3 & 4	53	47	5.3	38
	5+	48	40	5.3	13
	Total	101	44	5.3	53

¹ Average number of days postpartum on the day treatment with MGA began. Body condition scores were assigned one day prior to the day treatment with MGA was initiated using a scale 1 = emaciated to 9 = obese. Cyclicity was determined from 2 blood samples for progesterone obtained 10 days and 1 day prior to the day treatment with MGA was initiated.

Table 3 provides a summary of estrus response, synchronized conception and pregnancy, and final pregnancy rates for cows assigned to the two treatments. Estrus response was significantly higher among MGA[®]Select treated cows compared with the MGA-PG treated cows. Synchronized pregnancy rates were higher among the 5-year-old and older cows assigned to the MGA[®]Select treatment.

Table 3. Estrus response, synchronized conception and pregnancy rate, and final pregnancy rate at the end of the breeding period (Patterson et al., 2001). ^{a,b} Percentages within column and category with unlike superscripts are different (P<.05).								
Treatment	Age group (yrs)	Estrus response		Synchronized conception rate		Synchronized pregnancy rate		Final pregnancy
		(no.)	(%)	(no.)	(%)	(no.)	(%)	(no.) (%)
MGA-PG	2, 3 & 4	44/52	85	36/44	82	36/52	69	49/52 94
	5+	32/48	67	22/32	69	22/48	46 ^a	48/48 100
	Total	76/100	76 ^a	58/76	76	58/100	58	97/100 97
MGA-GnRH-PG	2, 3 & 4	46/53	87	33/46	72	33/53	62	51/53 96
	5+	42/48	88	34/42	81	34/48	71 ^b	47/48 98
	Total	88/101	87 ^b	67/88	76	67/101	66	98/101 97

The objective of a second experiment during the spring 2000 breeding season was to determine if MGA pretreatment could improve conception rates following a GnRH-PG-GnRH protocol (Perry et al., 2001). Cows from two University of Missouri herds

[Greenley Farm (n= 90); South Farm (n=137)] were assigned by age and days postpartum to one of two treatments. Control and MGA-treated (Figure 6) cows were fed a supplement carrier with or without MGA for 14 days. GnRH was administered to all cows 12 days after MGA or carrier withdrawal and 7 days prior to PG. All animals were administered GnRH and artificially inseminated 72 hours after PG.

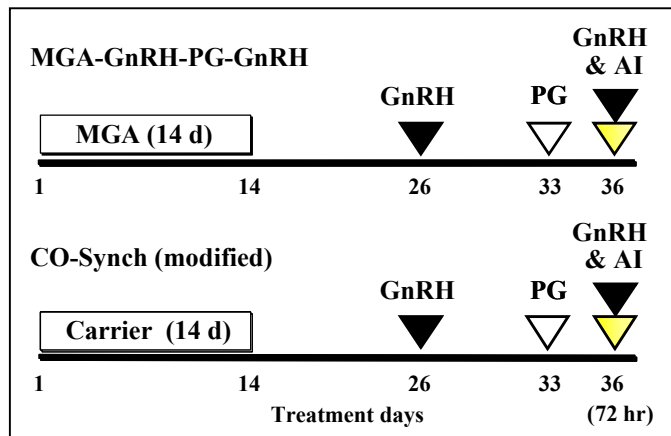


Figure 6. Treatment schedules and timing of fixed-time insemination for MGA-treated and Control (modified CO-Synch) cows (Perry et al., 2001).

Pregnancy rates to fixed-time AI were determined 50 days after insemination (Table 4). There was no difference between treatments at location 1 [MGA = 58% (26/45); Control = 51% (23/45)]. However, there was a difference ($P < .03$) in pregnancy rate to fixed-time AI between treatments at location 2 [MGA=63% (44/70); Control = 45% (30/67)]. Furthermore, when the data from both locations was combined the overall difference remained significant [MGA=70/115 (61%); Control=53/112 (47%); $P < .05$]. These data indicate that pregnancy rates resulting from fixed-time insemination are improved significantly when treatment with MGA precedes the GnRH-PG-GnRH protocol.

Table 4. Fixed-time AI and final pregnancy rates of MGA-treated and Control cows (Perry et al., 2001).						
Item	Location 1		Location 2		Total	
	No.	%	No.	%	No.	%
Pregnancy rate to fixed-time AI						
MGA-treated	26/45	58%	44/70	63% ^a	70/115	61% ^a
Control	23/45	51%	30/67	45% ^b	53/112	47% ^b
Final pregnancy rate						
MGA-treated	38/45	84%	64/70	91%	102/115	89%
Control	38/45	84%	59/67	88%	97/112	87%
^{a,b} Percentages within column and category with unlike superscripts are different ($P < .05$).						

METHOD 4: 7-11 SYNCH. Recently we developed an estrus synchronization protocol for beef cattle that was designed to: 1) shorten the feeding period of MGA without compromising fertility; and 2) improve synchrony of estrus by synchronizing development and ovulation of follicles from the first wave of development (Figure 7A; Kojima et al., 2000). This new treatment, 7-11 Synch, was compared with the GnRH-PG

protocol. Synchrony of estrus during the 24-hour peak response period (42 to 66-hour) was significantly higher among 7-11 Synch treated cows. Furthermore, the distribution of estrus was reduced from 144 hours for GnRH-PG treated cows to 60 hours for cows assigned to the 7-11 Synch treatment (Figure 7B; Kojima et al., 2000). The 7-11 Synch protocol resulted in a higher degree of estrus synchrony (91%) and greater AI pregnancy rate (68%) during a 24-hour peak response period compared to the GnRH-PG protocol (69% and 47%, respectively).

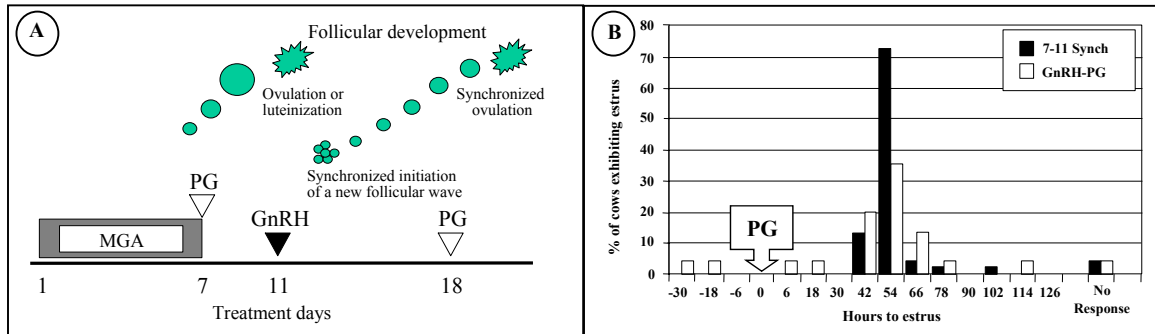


Figure 7A. Illustration of the treatment schedule and events associated with the 7-11 Synch protocol (Kojima et al., 2000). **Figure B.** Estrus response of cows treated with the 7-11 Synch or GnRH-PG protocols (Kojima et al., 2000).

ADDITIONAL CONSIDERATIONS. An additional consideration for Methods 2, 3 and 4 pertains to cows or heifers that fail to exhibit estrus after the last PG injection. In this case, cows or heifers would be re-injected with PG 11 to 14 days after the last injection of PG was administered. These females would then be observed for signs of behavioral estrus for an additional 6 to 7 days. This procedure would maximize efforts to inseminate as many females within the first 2 weeks of the breeding period as possible. Cows that were inseminated during the first synchronized period should not be re-injected with PG. In addition, the decision to use Methods 3 or 4 in heifers should be based on careful consideration of the heifer's age, weight, and pubertal status.

SUMMARY AND CONCLUSIONS. Expanded use of AI and/or adoption of emerging reproductive technologies for beef cows and heifers requires precise methods of estrous cycle control. Effective control of the estrous cycle requires the synchronization of both luteal and follicular functions. Efforts to develop a more effective estrus synchronization protocol have focused recently on synchronizing follicular waves by injecting GnRH followed 7 days later by injection of PG (Ovsynch, CO-Synch, Select Synch). A factor contributing to reduced synchronized pregnancy rates in dairy and beef cows treated with the preceding protocols is that 5 to 15% of cycling cows show estrus on or before PG injection. We developed new protocols for inducing and synchronizing a fertile estrus in postpartum beef cows and beef heifers in which the GnRH-PG protocol is preceded by either short- or long-term progestin treatment.

Although other types of progestin treatments (CIDR, PRID, or norgestomet) can be substituted in these estrus synchronization protocols, we chose to use MGA for the following reasons: a) MGA is economical to use (\approx 2 cents per animal per day to feed);

b) MGA was recently cleared for use in reproductive classes of beef and dairy cattle (Federal Register, 1997); c) methodology and understanding of the use of MGA is documented in the literature (Zimbelman, 1963; Zimbelman and Smith, 1966; Patterson et al., 1989), dating back as early as the 1960's; and d) MGA is easily administered in feed and does not require that animals be handled or restrained during administration. Perhaps more importantly, *MGA is currently the only progestin approved for use and available in the U. S.*, making research of methods to improve and broaden the scope of its use all the more significant.

Table 5 provides a summary of various estrus synchronization protocols for use in postpartum beef cows. The table includes estrus response for the respective treatments and the synchronized pregnancy rate that resulted. These data represent results from our own published work, in addition to unpublished data from DeJarnette and Wallace, Select Sires, Inc. The results shown in Table 5 provide evidence to support the sequential approach to estrus synchronization in postpartum beef cows we describe.

Our preliminary studies identified significant improvements in specific reproductive endpoints among cows that received MGA prior to the administration of PG compared with cows that received PG only, including increased estrus response and improved synchronized conception and pregnancy rates. More recently we observed a significant improvement in synchrony of estrus without compromising fertility in postpartum beef cows and beef heifers that were pretreated, either short- or long-term, with MGA prior to GnRH and PG. We propose the general hypothesis that progestin (MGA) treatment prior to the GnRH-PG estrus synchronization protocol will successfully: 1) induce ovulation in anestrus postpartum beef cows and peripubertal beef heifers; 2) reduce the incidence of a short luteal phase among anestrus cows induced to ovulate; 3) increase estrus response, synchronized conception and pregnancy rate; and 4) increase the likelihood of successful fixed-time insemination. Our data suggest that new methods of inducing and synchronizing estrus for postpartum beef cows and replacement beef heifers in which the GnRH-PG protocol is preceded by a progestin offer significant potential to more effectively synchronize estrus with resulting high fertility.

Table 5. Comparison of estrus response and fertility in postpartum beef cows after treatment with various estrus synchronization protocols.

Treatment	Estrus response		Synchronized pregnancy rate	
2 shot PG	241/422	57%	147/422	35%
Select Synch	353/528	67%	237/528	45%
MGA-PG 14-17 d	305/408	75%	220/408	54%
MGA-2 shot PG	327/348	93%	243/348	70%
MGA-PG 14-19 d	161/206	83%	130/206	63%
MGA [®] Select	174/204	85%	134/204	66%
MGA [®] Select + GnRH at AI	Fixed-time AI		70/115	61%
7-11 Synch	40/44	91%	30/44	68%

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